(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
12 July 2001 (12.07.2001)

PCT

(10) International Publication Number
WO 01/49268 A1

(51) International Patent Classification[7]: A61K 9/14, 9/127, 9/10, 9/19

(21) International Application Number: PCT/US00/35322

(22) International Filing Date:
21 December 2000 (21.12.2000)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
09/478,124 5 January 2000 (05.01.2000) US
09/703,484 31 October 2000 (31.10.2000) US

(71) Applicant: IMARX THERAPEUTICS, INC. [US/US]; 1635 East 18th Street, Tucson, AZ 85719 (US).

(72) Inventors: UNGER, Evan, C.; 6227 E. Miramar Drive, Tucson, AZ 85715 (US). ROMANOWSKI, Marek, J.; 605 E. Suffolk Drive, Tucson, AZ 85704 (US).

(74) Agents: REED, Dianne, E. et al.; Reed & Associates, 3282 Alpine Road, Portola Valley, CA 94028 (US).

(81) Designated States *(national)*: AU, CA, JP.

(84) Designated States *(regional)*: European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR).

Published:
— *With international search report.*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 01/49268 A1

(54) Title: PHARMACEUTICAL FORMULATIONS FOR THE DELIVERY OF DRUGS HAVING LOW AQUEOUS SOLUBILITY

(57) Abstract: Pharmaceutical formulations are provided that increase the systemic bioavailability of a drug that has low aqueous solubility. The drug is physically entrapped by a spatially stabilized matrix of a hydrophilic polymer, but is not covalently bound thereto. Phospholipid moieties are optionally conjugated to the hydrophilic polymer, and free phospholipids, stabilizing agents and/or other excipients may be incorporated into the formulations as well. Therapeutic methods are also provided, wherein a formulation of the invention is administered to a patient to treat a condition, disorder or disease that is responsive to a particular drug. Generally, administration is oral or parenteral.

PHARMACEUTICAL FORMULATIONS FOR THE DELIVERY OF DRUGS HAVING LOW AQUEOUS SOLUBILITY

TECHNICAL FIELD

The present invention relates generally to pharmaceutical formulations, and more particularly relates to pharmaceutical formulations for the delivery of water-insoluble or sparingly water-soluble drugs. The invention additionally relates to methods for using the novel formulations to administer such drugs in the treatment of disease. The invention has utility in the fields of pharmaceutical formulation, drug delivery, and medicine.

BACKGROUND ART

The formulation and administration of water-insoluble or sparingly water-soluble drugs is problematic because of the difficulty of achieving sufficient systemic bioavailability. Low aqueous solubility results not only in decreased bioavailability, but also in formulations that are insufficiently stable over extended storage periods. A classic example in this regard is paclitaxel, available commercially as Taxol® from Bristol-Myers Squibb. Although paclitaxel has been shown to exhibit powerful antineoplastic efficacy, particularly for cancers of the breast, ovaries and prostate gland, its use is limited in large part by the side effects of the solvent generally used for clinical administration, a mixture of Cremophor EL® (polyethoxylated castor oil) and ethanol. The amount of solvent that is required to deliver an effective dose of paclitaxel is substantial, and Cremophor has been shown to result in serious or fatal hypersensitivity episodes in laboratory animals (see, e.g., Lorenz et al. (1977) *Agents Actions* 7:63-67) as well as in humans (Weiss et al. (1990) *J. Clin. Oncol.* 8:1263-1268). Because of the undesirable physiologic reactions associated with paclitaxel-Cremophor formulations, patients are generally premedicated with corticosteroids and/or antihistamines. While premedication has proven to be somewhat effective, mild to moderate hypersensitivity is still a problem in a significant number of patients. Weiss et al., supra; see also Runowicz et al. (1993) *Cancer* 71:1591-1596.

Thus, extensive research has been conducted with the aim of producing an improved paclitaxel formulation having reduced toxicity. In particular, efforts have been directed toward (1) modifying the chemistry of the drug itself to make it more hydrophilic and (2) combining the drug with agents that produce water-soluble dispersions. Chemically modified paclitaxel analogs include sulfonated paclitaxel derivatives (see U.S. Patent No. 5,059,699), amino acid esters (Mathew et al. (1992) *J. Med. Chem.* 3B:145-151) as well as covalent conjugates of paclitaxel and polyethylene glycol (U.S. Patent No. 5,648,506 to Desai et al.; Liu et al. (1999) *J. Polymer Sci., Part A - Polymer Chem.* 37:3492-3503). For the most part, however, research has focused on entrapment of the drug in vesicles or liposomes, and on the incorporation of surfactants into paclitaxel formulations.

Representative liposomal drug delivery systems are described in U.S. Patent Nos. 5,395,619, 5,340,588 and 5,154,930. Liposomes, as is well known in the art, are vesicles comprised of concentrically ordered lipid bilayers that encapsulate an aqueous phase. Liposomes form when phospholipids, amphipathic compounds having a polar (hydrophilic) head group covalently bound to a long-chain aliphatic (hydrophobic) tail, are exposed to water. That is, in an aqueous medium, phospholipids aggregate to form a structure in which the long-chain aliphatic tails are sequestered within the interior of a shell formed by the polar head groups. Unfortunately, use of liposomes for delivering many drugs has proven unsatisfactory, in part because liposome compositions are, as a general rule, rapidly cleared from the bloodstream. Finally, even if satisfactory liposomal formulations could be prepared, it may still be necessary to use some sort of physical release mechanism so that the vesicle releases the active agent in the body before it is taken up by the liver and spleen.

Encasement of paclitaxel microcrystals in shells of biocompatible polymeric materials is described in U.S. Patent No. 6,096,331 to Desai et al. However, as crystals of hydrophobic drugs may be difficult to dissolve, the rate of drug release in these formulations is hard to control.

Incorporation of surfactants into paclitaxel formulations as described, for example, in International Patent Publication No. WO 97/30695, is also problematic. Surfactants tend to alter the chemistry of a pharmaceutical formulation such that the effective ratio of drug to inactive ingredients is lowered, resulting in the need to increase dosage volume and/or administration time. Additionally, formulations that employ surfactants readily dissociate

upon dilution, e.g., following intravenous injection, resulting in premature drug release. Also, many surfactants are considered unsuitable for parenteral drug administration because of their interaction with cellular membranes.

Accordingly, there is a need in the art for a pharmaceutical formulation that is suitable for administration of a water-insoluble or sparingly water-soluble drug such as paclitaxel or the like, wherein (1) the formulation is optimized such that the amount of drug administered is maximized while undesirable side effects are minimized, (2) the rate of drug release can be precisely controlled, (3) no surfactants are necessary, (4) no liposomes or other vesicles are required, (5) premedication is unnecessary, and (6) the formulation displays excellent stability over extended storage periods.

DISCLOSURE OF THE INVENTION

It is accordingly a primary object of the invention to address the above-mentioned needs in the art by providing a pharmaceutical formulation effective to deliver a drug having low aqueous solubility.

It is another object of the invention to provide a therapeutic method wherein the aforementioned formulation is administered to a patient to treat a condition, disease or disorder for which the drug is indicated.

It is an additional object of the invention to provide such a method wherein the drug is an anticancer agent and the patient is suffering from cancer.

It is a further object of the invention to provide a method for administering a drug so as to enhance the systemic bioavailability thereof.

Additional objects, advantages and novel features of the invention will be set forth in part in the description which follows, and in part will become apparent to those skilled in the art upon examination of the following, or may be learned by practice of the invention.

In one aspect of the invention, then, a pharmaceutical formulation is provided that comprises: (a) a matrix of a spatially stabilized hydrophilic polymer that is optionally covalently bound (or "conjugated") to a phospholipid moiety; (b) a drug that is physically entrapped within the matrix but not covalently bound thereto, wherein the drug has greater solubility in polyethylene glycol 400 than in water; (c) an optional stabilizing agent, (d) an optional targeting ligand, and (e) an optional excipient. A variety of hydrophilic polymers

may be employed, although polyethylene glycol and poly(ethylene oxide-co-propylene oxide) are preferred. Preferred drugs are those for which systemic bioavailability can be enhanced by increasing the solubility of the drug in an aqueous vehicle; generally, although not necessarily, such drugs are hydrophobic, i.e., water insoluble or sparingly water-soluble. Also preferred are drugs for which a sustained release drug delivery system is desirable, i.e., drugs that are administered to patients on an ongoing, scheduled basis. The stabilizing agents are generally polymers containing hydrophobic and hydrophilic areas, proteins are preferred. Suitable excipients include free phospholipids, which may or may not be the same as the phospholipid moieties conjugated to the hydrophilic polymer. The formulation may be in lyophilized form, which is advantageous for storage stability.

In another aspect of the invention, a pharmaceutical formulation is provided that comprises an aqueous suspension of (a) drug-containing particles having an average size in the range of approximately 1 nm to 500 μm, comprised of (i) a matrix of a spatially stabilized hydrophilic polymer that is optionally covalently bound to a phospholipid moiety, (ii) a drug that is physically entrapped within the matrix but not covalently bound thereto, wherein the drug has greater solubility in polyethylene glycol 400 than in water, optionally (iii) a stabilizing agent, optionally (iv) a targeting ligand, and optionally (v) an excipient, in (b) an aqueous vehicle. The aqueous vehicle may be, for example, water, isotonic saline solution or phosphate buffer, and may be instilled with an acoustically active gas to facilitate ultrasound imaging and ultrasonic cavitation for local drug release with ultrasound.

In still another aspect of the invention, a method is provided for delivering a drug to a mammalian individual to achieve a desired therapeutic effect, wherein the method involves administering to the individual a therapeutically effective amount of a formulation of the invention, e.g., orally or parenterally.

In a related aspect of the invention, a method is provided for treating an individual suffering from cancer, comprising parenterally administering to the patient a surfactant-free formulation of: (a) drug-containing particles comprised of (i) a matrix of a spatially stabilized hydrophilic polymer that is optionally covalently bound to a phospholipid moiety, (ii) an anticancer agent that is entrapped by but not covalently bound to the hydrophilic polymer, wherein the anticancer agent is selected from the group consisting paclitaxel, docetaxel, camptothecin, and derivatives and analogs thereof, (iii) a stabilizing agent, optionally (iv) a

stabilizing agent; optionally (v) a targeting ligand; and optionally (vi) an excipient selected from the group consisting of a free phospholipid, a saccharide, a liquid polyethylene glycol, propylene glycol, glycerol, ethyl alcohol, other polyhydroxyalcohols, and combinations thereof; in (b) an aqueous vehicle suitable for parenteral drug administration.

In another related aspect of the invention, an alternative method is provided for treating an individual suffering from cancer, comprising orally administering to the individual a pharmaceutical formulation of: (a) drug-containing particles comprised of (i) a matrix of a spatially stabilized hydrophilic polymer that is optionally covalently bound to a phospholipid moiety, (ii) an anticancer agent that is entrapped by but not covalently bound to the hydrophilic polymer, wherein the anticancer agent is selected from the group consisting of paclitaxel, docetaxel, camptothecin, and derivatives and analogs thereof, (iii) an effective amount of a P-glycoprotein inhibitor, optionally (iv) a stabilizing agent, optionally (v) a targeting ligand, and optionally (vi) an excipient selected from the group consisting of a free phospholipid, a saccharide, a liquid polyethylene glycol, propylene glycol, glycerol, ethyl alcohol, other polyhydroxyalcohols, and combinations thereof; in (b) an aqueous vehicle suitable for oral drug administration.

In yet another aspect of the invention, an improved method is provided for administering a drug so as to enhance the bioavailability thereof, wherein the improvement comprises administering the drug in a pharmaceutical formulation comprised of (a) a matrix of a spatially stabilized hydrophilic polymer that is optionally covalently bound to a phospholipid moiety, (b) a drug that is physically entrapped within the matrix but not covalently bound thereto, wherein the drug is water insoluble or sparingly water soluble, optionally (c) a stabilizing agent, optionally a targeting ligand, and optionally (e) an excipient selected from the group consisting of a free phospholipid, a saccharide, a liquid polyethylene glycol, propylene glycol, glycerol, ethyl alcohol, other polyhydroxyalcohols, and combinations thereof, wherein the formulation is free of surfactants.

The present invention is based on the formation of a noncovalent complex of drug molecules with a hydrophilic polymer and an optional biocompatible stabilizing agent. This drug/polymer complex allows for the formation of an aqueous suspension of nanoparticles of the complex without requiring chemical modification of the drug. This technology can be applied to many drugs having poor solubility in water, e.g., paclitaxel. Problems related to

stability, toxicity of the carrier, and large injection volume of currently available formulations of paclitaxel are well documented. Nanoparticle solubilization technology enables the preparation of paclitaxel formulations with decreased toxicity and improved efficacy.

We have discovered that a unique class of nanoparticles ranging from about 1 nm to about 500-1000 μm, preferably from about 1 nm to about 500 μm, that can be stabilized with a stabilizing agent to form nanoparticles having diameters ranging from about 1nm to about 300 nm, preferably from about 20 nm to about 100 nm. The resulting nanoparticles are biocompatible and highly useful for drug delivery. The drug delivery is preferably via IV injection but the technology has applications for oral, subcutaneous, e.g., sustained release, and pulmonary delivery. For IV delivery, the nanoparticles decrease toxicity of the therapeutic agents such as paclitaxel. Larger doses of the active agents can therefore be administered via IV, allowing for higher blood levels of the therapeutic agent yielding greater efficacy. For oral applications, the nanoparticles improve dispersal of insoluble drugs and increase uptake from the gastrointestinal tract. For sustained release applications, the nanoparticles can be formulated into gels, powders or suspensions. For pulmonary applications, the nanoparticles' small effective hydrodynamic radii improves delivery of therapeutic agents into the distal airways, such as the alveoli, thereby allowing systemic delivery of bioactive agents via the pulmonary route.

The size of the particles within the formulation helps to control dispersal of the drug and drug release. Surprisingly, stabilized and unstabilized drug/polymer complexes have improved solubility and drug release properties compared to crystalline forms of the drug. The rate of release of drug/polymer complexes can be fine-tuned by optionally including a stabilizing agent and by varying the nature of the drug complex. For example, branched polyethylene glycol (PEG) is a soluble polymer that is capable of forming complexes with certain hydrophobic drugs. Once in the body, the PEG will eventually dissolve, releasing the complexed drug. The rate of drug release can be modified by varying the conditions and parameters of complex formation, e.g., ratios of PEG to drug, chemical structure of the PEG, and the amount and type of stabilizing agent. Hydrolyzable bonds may also be incorporated into the hydrophilic polymer and/or the stabilizing agent to accelerate drug release, and pH-responsive groups may be used to increase drug release at a desired pH.

BRIEF DESCRIPTION OF THE DRAWINGS

FIG. 1 schematically illustrates a formulation of the invention in which a drug molecule (paclitaxel) is "entrapped" within a spatially stabilized matrix of phospholipid-conjugated polyethylene glycol (e.g., dipalmitoyl phosphatidylethanolamine [DPPE] conjugated to polyethylene glycol).

FIG. 2 schematically illustrates an alternative formulation of the invention in which a drug is entrapped within a spatially stabilized matrix of a highly branchedpolyethylene glycol molecule.

FIG. 3 schematically illustrates another alternative formulation of the invention in which a drug is entrapped within a spatially stabilized matrix formed by star polyethylene glycol.

FIG. 4 schematically illustrates still another alternative formulation of the invention in which a drug is entrapped within a spatially stabilized matrix of lower molecular weight, branched polyethylene glycol.

FIG. 5 presents sizing data of paclitaxel nanoparticles stabilized with human serum albumin.

FIG. 6 presents the body weights of nude mice treated with nanoparticulate formulations of paclitaxel in an MTD (maximally tolerated dose study).

FIG. 7 relates tumor growth in nude mice following single doses at 2/3^{rds} MTD of Taxol and the formulation of the invention.

FIG. 8 relates tumor growth in nude mice following single doses at full MTD of Taxol and the formulation of the invention.

FIG. 9 shows a branched PEG molecule with 4 arms stabilizing 4 molecules of a therapeutic agent. The number of therapeutic molecules stabilized per molecule of branched PEG will vary depending upon the molecular weight of each arm the PEG, the molecular weight of the drug, the formulation and the intended application. In the figure, the numeral "1" refers to a drug molecule, while the numeral "2" refers to the polymer.

FIG. 10 shows a modified branched PEG with 4 arms stabilizing 4 molecules of a therapeutic agent. In this case the core of the branched molecule has been substituted with another polymer more hydrophobic than PEG such as polypropylene glycol. The hydrophobic core favors partitioning of hydrophobic drugs into the core and stabilization of

drug within the interior of the branched molecule. Depending upon the ratio of drug to branched molecule, the PEG comprised outer arms may be free to move as would a hydrated PEG molecule. In this case, a single branched molecule may form a stable association with a plurality of drug molecules, and do not aggregate to form a particle. In the figure, the numeral "1" refers to a drug molecule, the numeral "2" refers to hydrophilic polymer in outer part of arms and the numeral "3" refers to the more hydrophobic core polymer.

FIG. 11 shows a modified branched PEG molecule similar to Figure 10, except in this case the termini of the outermost PEG groups have been modified to covalently bind targeting ligands. As shown in this Figure, the branched PEG molecule may bind more than one type of targeting ligand. The bound targeting ligands facilitate drug delivery to specific cells bearing receptors for the particular targeting ligands. In the figure, the numeral "1" refers to a drug molecule, the numeral "2" refers to hydrophilic polymer in outer part of arms, the numeral "3" refers to the more hydrophobic core polymer and the numeral "4" refers to the targeting ligands (two different types are shown here).

MODES FOR CARRYING OUT THE INVENTION

I. DEFINITIONS AND OVERVIEW:

It is to be understood that unless otherwise indicated, this invention is not limited to specific active agents, hydrophilic polymers, phospholipids, excipients, methods of manufacture or the like, as such may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only and is not intended to be limiting.

It must be noted that, as used in the specification and the appended claims, the singular forms "a," "an" and "the" include plural referents unless the context clearly dictates otherwise. Thus, for example, reference to "an active agent" or "a drug" in a formulation means that more than one active agent can be present, reference to "a hydrophilic polymer" includes combinations of hydrophilic polymers, reference to "a phospholipid" includes mixtures of phospholipids, and the like.

In this specification and in the claims that follow, reference will be made to a number of terms that shall be defined to have the following meanings:

By "pharmaceutically acceptable" is meant a material that is not biologically or otherwise undesirable, i.e., the material may be administered to an individual along with the selected active agent without causing any undesirable biological effects or interacting in a deleterious manner with any of the other components of the pharmaceutical composition in which it is contained.

"Pharmaceutically or therapeutically effective dose or amount" refers to a dosage level sufficient to induce a desired biological result. That result can be alleviation of the signs, symptoms, or causes of a disease, or any other desired alteration of a biological system.

The term "treat" as in "to treat a disease" is intended to include any means of treating a disease in a mammal, including (1) preventing the disease, i.e., avoiding any clinical symptoms of the disease, (2) inhibiting the disease, that is, arresting the development or progression of clinical symptoms, and/or (3) relieving the disease, i.e., causing regression of clinical symptoms.

The terms "disease," "disorder" and "condition" are used interchangeably herein to refer to a physiological state that may be treated using the formulations of the invention.

The terms "drug," "active agent" and "therapeutic agent" are used interchangeably herein to refer to a chemical material or compound which, when administered to an organism (human or animal), induces a desired pharmacologic effect. Included are analogs and derivatives (including salts, esters, prodrugs, and the like) of those compounds or classes of compounds specifically mentioned which also induce the desired pharmacologic effect.

The number given as the "molecular weight" of a compound, as in the molecular weight of a hydrophilic polymer such as polyethylene glycol, refers to weight average molecular weight M_w.

The "solubility" of a compound refers to its solubility in the indicated liquid determined under standard conditions, e.g., at room temperature (typically about 25°C), atmospheric pressure, and neutral pH.

The term "hydrophobic" is used to refer to a compound having an octanol:water partition coefficient (at room temperature, generally about 23°C) of at least about 8:1, preferably at least about 10:1, more preferably 20:1 or higher. "Hydrophobic" drugs are sometimes referred to herein as "water insoluble" or "sparingly water soluble," or as having "low aqueous solubility." The term "hydrophilic" refers to a material that is not hydrophobic.

In referring to chemical compounds herein, the following definitions apply:

The term "alkyl" refers to a branched or unbranched saturated hydrocarbon group of 1 to 24, typically 1 to 18, carbon atoms, such as methyl, ethyl, *n*-propyl, isopropyl, *n*-butyl, isobutyl, *t*-butyl, octyl, decyl, tetradecyl, hexadecyl, eicosyl, tetracosyl and the like, as well as cycloalkyl groups such as cyclopentyl, cyclohexyl and the like.

The term "aryl" refers to an aromatic species containing 1 to 3 aromatic rings, either fused or linked, and either unsubstituted or substituted with one or more substituents. Preferred aryl substituents contain one aromatic ring or two fused aromatic rings.

The term "acyl" refers to a group having the structure R(CO)- wherein R is alkyl or aryl as defined above.

"Optional" or "optionally" means that the subsequently described circumstance may or may not occur, so that the description includes instances where the circumstance occurs and instances where it does not.

II. Formulations:

The pharmaceutical formulations of the invention are advantageously used to deliver any drug whose systemic bioavailability (including oral bioavailability) can be enhanced by increasing the solubility of the drug in water. Thus, the drugs that are preferred for use in conjunction with the present invention are generally hydrophobic in nature, tending toward low aqueous solubility. The invention incorporates such drugs in a composition comprised of a matrix of a spatially stabilized hydrophilic polymer that physically entraps and thereby immobilizes the drug, but does not covalently bind thereto. The composition may additionally comprise a stabilizing agent that further stabilizes the hydrophilic polymer/drug complex and is useful in forming nanoparticulate-stabilized complexes.

A. The Hydrophilic Polymer

The hydrophilic polymer of the present formulations is spatially stabilized so as to facilitate physical entrapment and thus immobilization of the active agent; that is, the "spatially stabilized" hydrophilic polymer forms a matrix or three-dimensional structure in which discrete regions of drug are dispersed. By "spatially stabilized" is meant that the relative orientation of the drug in the polymer matrix is fixed in three-dimensional space

without directional specification. Generally, although not necessarily, the "spatially stabilized" matrix is sterically constrained. Any polymer that can form such a matrix can be used in conjunction with the invention, providing that the polymer is sufficiently hydrophilic to increase the aqueous solubility of the entrapped drug. It is preferred that the polymer is not cross-linked.

Examples of suitable hydrophilic polymers include, but are not limited to, polyethylene glycol, polypropylene glycol, polyvinyl alcohol, polyvinyl pyrrolidone, polylactide, poly(lactide-co-glycolide), polysorbate, polyethylene oxide, polypropylene oxide, poly(ethylene oxide-co-propylene oxide), poly(oxyethylated) glycerol, poly(oxyethylated) sorbitol, poly(oxyethylated) glucose), and derivatives, mixtures and copolymers thereof. Examples of suitable derivatives include those in which one or more C-H bonds, e.g., in alkylene linking groups, are replaced with C-F bonds, such that the polymers are fluorinated or even perfluorinated.

The preferred hydrophilic polymer for use in the present formulations is polyethylene glycol (PEG) or a copolymer thereof, e.g., polyethylene glycol containing some fraction of other monomer units (e.g., other alkylene oxide segments such as propylene oxide), with polyethylene glycol itself most preferred. In order to form the spatially stabilized matrix, the polyethylene glycol used is either branched PEG (including "dendrimeric" PEG, i.e., higher molecular weight, highly branched PEG) or star PEG, optionally conjugated to a phospholipid moiety as will be discussed below. Covalent conjugates of linear PEG and phospholipids may also be used, since such conjugates can give rise to a spatially stabilized matrix, as the hydrophobic chains of the phospholipids will tend to associate in an aqueous medium. See FIG. 1, which schematically illustrates a formulation in which a drug is entrapped within a spatially stabilized matrix of phospholipid-conjugated linear PEG. Combinations of different types of PEG (e.g., branched PEG and linear PEG, star PEG and linear PEG, branched PEG and phospholipid-conjugated linear PEG, etc.) may also be employed.

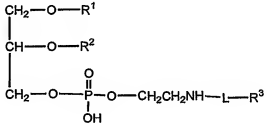
Branched PEG molecules will generally although not necessarily have a molecular weight in the range of approximately 1000 to 600,000 Daltons, more typically in the range of approximately 2000 to 10,000 Daltons, preferably in the range of approximately 20,000 to 40,000 Daltons. Branched PEG is commercially available, such as from Nippon Oil and Fat

(NOF Corporation, Tokyo, Japan) and from Shearwater Polymers (Huntsville, Alabama), or may be readily synthesized by polymerizing lower molecular weight linear PEG molecules (i.e., PEG 2000 or smaller) functionalized at one or both termini with a reactive group. For example, branched PEG can be synthesized by solution polymerization of lower molecular weight PEG acrylates (i.e., PEG molecules in which a terminal hydroxyl group is replaced by an acrylate functionality -O-(CO)-CH=CH_2) or methacrylates (similarly, PEG molecules in which a hydroxyl group is replaced by a methacrylate functionality -O-(CO)-C(CH_3)=CH_2) in the presence of a free radical polymerization initiator such as 2,2'-azobisisobutyronitrile (AIBN). If desired, mixtures of PEG monoacrylates or monomethacrylates having different molecular weights can be used in order to synthesize a branched polymer having "branches" or "arms" of differing lengths. Branched PEGs have 2 or more arms but may have as many as 1000 arms. The branched PEGs herein preferably have about 4 to 40 arms, more preferably about 4 to 10 arms, and most preferably about 4 to 8 arms. Higher molecular weight, highly branched PEG, e.g., branched PEG having a molecular weight of greater than about 10,000 and at least about 1 arm (i.e., one branch point) per 5000 Daltons, will sometimes be referred to herein as "dendrimeric" PEG. Such PEG is preferably formed by reaction of a hydroxyl-substituted amine such as triethanolamine with lower molecular weight PEG that may be linear, branched or star, to form a molecular lattice that serves as the spatially stabilized matrix and entraps the active agent to be delivered. Dendrimeric structures including dendrimeric PEG are described, *inter alia*, by Liu et al. (1999) *PSTT* 2(10):393-401. Formulations of the invention prepared with highly branched, high molecular weight dendrimeric PEG and with lower molecular weight branched PEG are schematically illustrated in FIGS. 2 and 4, respectively.

Star molecules of PEG are available commercially (e.g., from Shearwater Polymers, Huntsville, AL) or may be readily synthesized using living free radical polymerization techniques as described, for example, by Gnanou et al. (1988) *Makromol. Chem.* 189:2885-2892 and Desai et al., U.S. Patent No. 5,648,506. Star PEG generally has a central core of divinyl benzene or glycerol. Preferred molecular weights for star molecules of PEG useful herein are typically in the range of about 1000 to 500,000 Daltons, although molecular weights in the range of about 10,000 to 200,000 are preferred. A formulation of the invention that employs star PEG is schematically illustrated in FIG. 3.

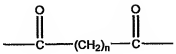
As explained above, conjugates of hydrophilic polymers and phospholipids, particularly PEG-phospholipid conjugates (also termed "PEGylated" phospholipids), are also useful in the present formulations. The polyethylene glycol in the PEGylated phospholipids may be branched, star or linear. Conjugates of linear PEG and phospholipids, if used, will generally although not necessarily employ PEG have a molecular weight in the range of approximately 1000 to 50,000 Daltons, preferably in the range of approximately 1000 to 40,000 Daltons. It will be appreciated by those skilled in the art that the aforementioned molecular weight ranges correspond to a polymer containing approximately 20 to 1000 ethylene oxide units, preferably about 20 to 2000 ethylene oxide units. The phospholipid moiety that is conjugated to the PEG may be anionic, neutral or cationic, of naturally occurring or synthetic origin, and normally comprises a diacyl phosphatidylcholine, a diacyl phosphatidylethanolamine, a diacyl phosphatidylserine, a diacyl phosphatidylinositol, a diacyl phosphatidylglycerol, or a diacyl phosphatidic acid, wherein each acyl moiety can be saturated or unsaturated and will generally be in the range of about 10 to 22 carbon atoms in length. Preferred compounds are polymer-conjugated diacyl phosphatidyl-ethanolamines having the structure of formula (I)

(I)



wherein R^1 and R^2 are the acyl groups, R^3 represents the hydrophilic polymer, e.g., a polyalkylene oxide moiety such as poly(ethylene oxide) (i.e., polyethylene glycol), poly(propylene oxide), poly(ethylene oxide-co-propylene oxide) or the like (for linear PEG, R^3 is -O-$(CH_2CH_2O)_n$-H), and L is an organic linking moiety such as a carbamate, an ester, or a diketone having the structure of formula (II)

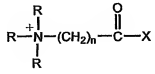
(II)



wherein n is 1, 2, 3 or 4. Preferred unsaturated acyl moieties are esters formed from oleic and linoleic acids, and preferred saturated acyl moieties are palmitate, myristate and stearate. Particularly preferred phospholipids for conjugation to linear, branched or star PEG herein are dipalmitoyl phosphatidylethanolamine (DPPE) and 1-palmitoyl-2-oleyl phosphatidylethanolamine (POPE).

The conjugates may be synthesized using art-known methods such as described, for example, in U.S. Patent No. 4,534,899 to Sears. That is, synthesis of a PEG-phospholipid conjugate or a conjugate of a phospholipid and an alternative hydrophilic polymer may be carried out by activating the polymer to prepare an activated derivative thereof, having a functional group suitable for reaction with an alcohol, a phosphate group, a carboxylic acid, an amino group or the like. For example, a polyalkylene oxide such as PEG may be activated by the addition of a cyclic polyacid, particularly an anhydride such as succinic or glutaric anhydride (ultimately resulting in the linker of formula (II) wherein n is 2 or 3, respectively). The activated polymer may then be covalently coupled to the selected phosphatidylalkanolamine, such as phosphatidylethanolamine, to give the desired conjugate.

The hydrophilic polymer may be modified in one or more ways. For drugs that are ionized at physiological pH, charged groups may be inserted into the hydrophilic polymer in order to modify the sustained release profile of the formulation. To reduce the rate of drug release and thereby provide sustained delivery over a longer time period, negatively charged groups such as phosphates and carboxylates are used for cationic drugs, while positively charged groups such as quaternary ammonium groups are used in combination with anionic drugs. A terminal hydroxyl group of a hydrophilic polymer such as PEG may be converted to a carboxylic acid or phosphate moiety by using a mild oxidizing agent such as chromic (VI) acid, nitric acid or potassium permanganate; a preferred oxidizing agent is molecular oxygen used in conjunction with a platinum catalyst. Introduction of phosphate groups may be carried out using a phosphorylating reagent such as phosphorous oxychloride ($POCl_3$) (see Example 11). Terminal quaternary ammonium salts may be synthesized, for example, by reaction with a moiety such as



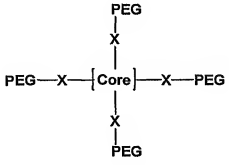
wherein R is H or lower alkyl (e.g., methyl or ethyl), n is typically 1 to 4, and X is an activating group such as Br, Cl, I or an -NHS ester. If desired, such charged polymers may be used to form higher molecular weight aggregates by reaction with a polyvalent counter ion.

Other possible modifications to the hydrophilic polymer include, but are not limited to, the following. A terminal hydroxyl group of a PEG molecule may be replaced by a thiol group using conventional means, e.g., reacting hydroxyl-containing PEG with a sulfur-containing amino acid such as cysteine, using a protected and activated amino acid. Such "PEG-SH" is also commercially available, for example from Shearwater Polymers. Alternatively, a mono(lower alkoxy)-substituted PEG such as monomethoxy polyethylene glycol (MPEG) may be used instead of polyethylene glycol *per se*, so that the polymer terminates with a lower alkoxy substituent (such as a methoxy group) rather than with a hydroxyl group. Similarly, PEG amine may be used in lieu of PEG so that a terminal amine moiety $-NH_2$ is present instead of a terminal hydroxyl group.

In addition, as discussed above, the polymer may contain two or more types of monomers, as in a copolymer wherein propylene oxide groups ($-CH_2CH_2CH_2O-$) have been substituted for some fraction of ethylene oxide groups ($-CH_2CH_2O-$) in polyethylene glycol. Incorporating propylene oxide groups will tend to increase the stability of the spatially stabilized matrix that entraps the drug, thus decreasing the rate at which the drug is released in the body. The more hydrophobic the drug and the larger the fraction of propylene oxide blocks, the slower the drug release rate will be.

The hydrophilic polymer may also contain hydrolyzable linkages to enable hydrolytic degradation within the body and thus facilitate drug release from the polymeric matrix. Suitable hydrolyzable linkages include any intramolecular bonds that can be cleaved by hydrolysis, typically in the presence of acid or base. Examples of hydrolyzable linkages include, but are not limited to, those disclosed in International Patent Publication No. WO 99/22770 to Harris, such as carboxylate esters, phosphate esters, acetals, imines, ortho esters and amides. Other suitable hydrolyzable linkages include, for example, enol ethers, diketene acetals, ketals, anhydrides and cyclic diketenes. Formation of such hydrolyzable linkages within the hydrophilic polymer is conducted using routine chemistry known to those skilled in the art of organic synthesis and/or described in the pertinent texts and literature. For

example, carboxylate linkages may be synthesized by reaction of a carboxylic acid with an alcohol, phosphate ester linkages may be synthesized by reaction of a phosphate group with an alcohol, acetal linkages may be synthesized by reaction of an aldehyde and an alcohol, and the like. Thus, polyethylene glycol containing hydrolyzable linkages "X" might have the structure -PEG-X-PEG- or alternatively might be a matrix having the structure



wherein the core is hydrophobic molecule such as pentaerythritol, may be synthesized by reaction of various -PEG-Y molecules with -Core-Z or PEG-Z molecules wherein Z and Y represent groups located at the terminus of individual PEG molecules and are capable of reacting with each other to form the hydrolyzable linkage X.

Accordingly, it will be appreciated that the rate of drug release from the polymeric matrix can be controlled by adjusting the degree of branching of the hydrophilic polymer, by incorporating different types of monomer units in the polymer structure, by functionalizing the hydrophilic polymer with different terminal species (which may or may not be charged), and/or by varying the density of hydrolyzable linkages present within the polymeric structure.

As illustrated above, the branched PEG molecule may be modified to have a hydrophobic core. For example, if the central core is pentaerythritol, the innermost arms bound to the pentaerythritol may comprise a polymer more hydrophobic than PEG. Useful polymers to accomplish this include polypropylene glycol and polybutylene glycol. Useful monomers for constructing the inner, hydrophobic core structures of the arms include propylene oxide, butylene oxide, copolymers of the two, lactic acid and copolymers of lactic acid with glycolide (polylactide-co-glycolide and copolymers of the foregoing with

polyethylene glycol). The preferred materials for constructing an inner hydrophobic core include polypropylene glycol and copolymers of propylene oxide with ethylene oxide. Useful polymers for constructing the outer, peripheral parts of the arms include polyethylene glycol, polysialic acid and other hydrophilic polymers, with PEG most preferred. It is possible that a fraction of the monomers in the outer portion of a given arm of the carrier molecule may be replaced with PEG, but in this case, there will be substantially more of the hydrophilic monomer (e.g. ethylene oxide) than the hydrophobic monomer (e.g. propylene oxide).

The relative proportion of hydrophobic polymer within the branched polymer may vary from about 10 wt.% to about 90 wt.% on a weight/weight ratio, preferably from about 40 wt.% to about 60 wt.%. When more hydrophobic polymer is used this may increase the drug loading capacity of the branched molecule for hydrophobic drugs. A most preferred ratio is about 50 wt.% weight of hydrophobic polymer, e.g. polypropylene glycol, and 50 wt.% weight ratio of hydrophilic polymer (e.g. PEG) in the outer arms.

The branched molecules in the hydrophobic core and peripheral hydrophilic arms are thought to have a number of advantages for drug delivery. The hydrophobic core may better stabilize hydrophobic drugs within the branched molecule and, as the drug is stabilized within the core, the free arms of the PEG may be better able to maintain a random state in which the PEG molecules move freely within solution. The outer, hydrophilic PEG layer may act as a steric barrier, inhibiting or decreasing the aggregation of individual branched molecules into particles. Additionally, in instances when targeting ligands are attached to the termini of the peripheral hydrophilic arms, targeting is facilitated by the unencumbered and exposed nature of the outer PEG arms. As will be discussed further on, a wide variety of targeting ligands can be covalently bound to the free ends of the PEG. The hydrophobic and hydrophilic components of the arms may be linked together by a variety of different. Such linkers include ethers, amides, esters, carbamates, thioesters, disulfide bonds. In general, the linker employed is used to attain the desired drug delivery properties of the pharmaceutical formulation. Metabolizable bonds can be selected to improve excretion of the carrier molecule as well as to improve drug release.

As previously mentioned, the free ends of the hydrophilic portions of the branches can be substituted with one or more targeting ligands per carrier molecule. More than one

kind of targeting ligand may be bound to each carrier molecule to facilitate binding to a target cell bearing more than one kind of receptor. A wide variety of ligands may be used in this regard. Exemplary targeting ligands include, for example, proteins, peptides, polypeptides, antibodies, antibody fragments, glycoproteins, carbohydrates, hormones, hormone analogs, lectins, amino acids, sugars, saccharides, vitamins, steroids, steroid analogs, enzyme cofactors, bioactive agents, and genetic material.

Generally speaking, peptides that are particularly useful as targeting ligands include natural, modified natural, or synthetic peptides that incorporate additional modes of resistance to degradation by vascularly circulating esterases, amidases, or peptidases. While many targeting ligands may be derived from natural sources, some may be synthesized by molecular biological recombinant techniques and other ligands may be synthetic in origin. Peptides may be prepared by a variety of different combinatorial chemistry techniques as are now known in the art. One very useful method of stabilizing a peptide moiety incorporates the use of cyclization techniques. For example, end-to-end cyclization, whereby the carboxy terminus is covalently linked to the amine terminus via an amide bond, may be useful to inhibit peptide degradation and increase circulating half-life. Side chain-to-side chain cyclization may also be particularly useful in inducing stability. In addition, an end-to-side chain cyclization may be a useful modification as well. The substitution of an L-amino acid for a D-amino acid in a strategic region of the peptide may also provide resistance to biological degradation. Suitable targeting ligands, and methods for their preparation will be readily apparent to one skilled in the art. Although the lengths of the peptides utilized as targeting ligands may vary, peptides having from about 5 to about 15 amino acid residues are generally preferred.

Antibodies may be used as whole antibodies or as antibody fragments, e.g., Fab or Fab'2, either of natural or recombinant origin. The antibodies of natural origin may be of animal or human origin, or may be chimeric (e.g., mouse/human). Human recombinant or chimeric antibodies are preferred and fragments are preferred to whole antibodies. Immunoglobulins typically comprise a flexible "hinge" region. See, e.g., "Concise Encyclopedia of Biochemistry," Second Edition, Walter de Gruyter & Co., pp. 282-283 (1988). Antibodies may be linked to the termini of the outer hydrophilic arms using the thiols of this "hinge" region. This is a preferred region for coupling antibodies, as the potential

binding site may be remote from the antigen-recognition site. Generally speaking, it may be difficult to utilize the thiols of the hinge group unless they are adequately prepared. As described in Shahinian and Salvias (1995) *Biochimica et Biophysica Acta* 1239:157-167, it may be desirable to reduce the thiol groups so that they are available for coupling, e.g., to maleimide derivatized linking groups. Examples of reducing agents that may be used include ethanedithiol, mercaptoethanol, mercaptoethylamine or the more commonly used dithiothreitol, commonly referred to as Cleland's reagent. However, care should be exercised when utilizing certain reducing agents, such as dithiothreitol, as overreduction may compromise the activity or binding capacity of the targeting ligand. See, e.g., Shahinian and Salvias, *supra*.

Antibody fragments, such as F(ab')2, may be prepared by incubating the antibodies with pepsin (60 μg/ml) in 0.1 M sodium acetate (pH 4.2) for 4 h at 37°C. Digestion may be terminated by the addition of 2 M Tris (pH 8.8) to a final concentration of 80 mM. The fragments may then be obtained by centrifugation. The supernatant may be dialyzed at 4°C against 150 mM NaCl, 20 mM phosphate at pH 7.0. Undigested IgG may be removed by chromatographic methods. The antibody fragments may then be extensively degassing the solutions and purging with nitrogen prior to use. The F(ab')2 fragments may be provided at a concentration of 5 mg/ml and reduced under argon in 30 mM cysteine. Alternatively, cysteamine may be employed; 100 mM Tris, pH 7.6 may be used as a buffer for 15 min at 37°C. The solutions may then be diluted 2-fold with an equal volume of the appropriate experimental buffer and spun through a 0.4 ml spin column of Bio-Gel P-6DG. The resulting antibody fragments may be more efficient in their coupling to the outer arms.

The same procedure may also be employed with other macromolecules containing cysteine residues for coupling to the termini of the PEG arms. Also, peptides may be utilized, especially if they contain a cysteine residue. If the peptides have not been made fresh and there is a possibility of oxidation of cysteine residues within the peptide structure, it may be necessary to regenerate the thiol group using the approach outlined above.

In one embodiment of the invention, the attached targeting ligands may be directed toward lymphocytes that may be T–cells or B–cells, with T–cells being the preferred target. To select a class of targeted lymphocytes, a targeting ligand having specific affinity for that class may be preferably employed. For example, an anti CD–4 antibody may be used for

selecting the class of T–cells harboring CD–4 receptors, an anti CD–8 antibody may be used for selecting the class of T-cells harboring CD–8 receptors, an anti CD–34 antibody may be used for selecting the class of T-cells harboring CD–34 receptors, and so on. A lower molecular weight ligand may preferably be employed, e.g., Fab or a peptide fragment. For example, an OKT3 antibody or OKT3 antibody fragment may be used.

Another major area for targeted delivery preferably involves the interleukin–2 (IL–2) system. IL–2 is a T–cell growth factor generally produced following antigen- or mitogen-induced stimulation of lymphoid cells. Cell types that typically produce IL–2 include, for example, CD4+ and CD8+ T–cells and large granular lymphocytes, as well as certain T–cell tumors. Generally speaking, IL–2 receptors are glycoproteins that are expressed on responsive cells. They are notable in connection with the present invention because they are generally readily endocytosed into lysosomal inclusions when bound to IL–2.

In addition to IL-2 receptors, preferred targets include the anti-IL-2 receptor antibody, natural IL-2 and an IL-2 fragment of a 20-mer peptide or smaller generated by phage display that binds to the IL-2 receptor. In use, for example, IL–2 may be conjugated to stabilizing materials, for example, in the form of vesicles, and thus may mediate the targeting of cells bearing IL–2 receptors. Endocytosis of the ligand–receptor complex may then deliver the compound to be delivered to the targeted cell. Additionally, an IL-2 peptide fragment which has binding affinity for IL–2 receptors may be incorporated, for example, by attachment to the termini of a different outer arm either directly to a reactive moiety or via a spacer or linker molecule with a reactive end such as an amine, hydroxyl, or carboxylic acid functional group. Such linkers are well known in the art and may comprise from 3 to 20 amino acid residues. In addition, D–amino acids or derivatized amino acids may be used which avoids proteolysis in the target tissue.

Still other systems which may be used in the present invention include IgM–mediated endocytosis in B–cells or a variant of the ligand–receptor interactions described above wherein the T–cell receptor is CD2 and the ligand is lymphocyte function-associated antigen 3 (LFA–3), as described, for example, in Wallner et al. (1987) *J. Experimental Med.* 166:923-932. Targeting ligands derived or modified from human leukocyte origin, such as CD11a/CD18 and leukocyte cell surface glycoprotein (LFA-1), may also be used as these may bind to the endothelial cell receptor ICAM-1. The cytokine inducible member of the

immunoglobulin superfamily, VCAM-1, which is mononuclear leukocyte-selective, may also be used as a targeting ligand. VLA-4, derived from human monocytes, may be used to target VCAM-1.

Preferred targeting ligands in accordance with the present invention include, for example, Sialyl Lewis X, mucin, hyaluronic acid, LFA-1, N-formal peptide, C5a, leukotriene B4, platelet activating factor, IL-8/NAP-1, CTAP-III, RANTES, and I-309. In addition, the integrins may be used as targeting ligands for targeting VLA-4, fibrinogen, von Willebrand factor, fibronectin, vitronectin, VCAM-1 and CD49d/CD29. A particularly preferred targeting ligand may be Sialyl Lewis X which binds to P-selectin and which has the following sequence: αNeu5Ac(2→3)βGal(1→4)[αFuc(1→3)]-βGlcNAc-OR wherein R is an aglycone having at least one carbon atom.. P-selectin may be a preferred target because it typically localizes on the luminal side of endothelium during inflammation, but generally not in non-inflammatory synovia where it is generally cytoplasmic only.

Other preferred targeting ligands include, for example, antibodies directed to autoantigens on T-cell receptors. Peptides having the amino acid sequences Leu Leu Ile Tyr Phe Asn Asn Asn Val Pro Ile Asp Asp Ser Gly Met (SEQ ID NO:1) and Lys Ile Gln Pro Ser Glu Pro Arg Asp Ser Ala Val Tyr Phe Cys Ala (SEQ ID NO:2) can be used to produce antibodies that may bind to the autoantigen portions of T-cell receptors. In addition, antibodies to additional T-cell and B-cell receptors may be used as targeting ligands. T- and B-cell receptors involved in inflammation and rheumatoid arthritis are described in Struyk et al. (1995), "T Cell Receptors in Rheumatoid Arthritis," *Arthritis & Rheumatism* 38:577-89; Marchalonis et al. (1994), "Naturally Occurring Human Autoantibodies to Defined T-Cell Receptor and Light Chain Peptides," *Immunobiol. Proteins Peptides*, pp. 135-45; Dedeoglu et al. (1993), "Lack of Preferential Usage in Synovial T Cells of Rheumatoid Arthritis Patients," *Immunol. Res.* 12:12-20; " Marchalonis, et al. (1993), "Human Autoantibodies to a Synthetic Putative T Cell Receptor Beta-Chain Regulatory Idiotype: Expression in Autoimmunity and Aging," *Exp. Clin. Immunogenet.* 10:1-15; Dehghanpisheh et al. (1996), "Peptide Epitope Binding Specificity and VK and VH Gene Usage in a Monoclonal IgM Natural Autoantibody to T Cell Receptor CDR1 from a Viable Motheaten Mouse," *Immunological Invest.* 25:241-52; Schluter et al. (1995), "Autoregulation of TCR V Region Epitopes in Autoimmune Disease," *Immunobiol. Proteins Peptides VIII*, pp.231-36; Lake, et al. (1995),

"Characterization of Autoantibodies Directed Against T Cell Receptors," *Immunobiol. Proteins Peptides VIII*, pp.223-29; Lake et al. (1994), "Construction and Serological Characterization of a Recombinant Human Single Chain T Cell Receptor," *Biochem. Biophys. Res. Comm.* 201:1502-1509; Lanchbury et al. (1995), "T Cell Receptor Usage in Rheumatoid Arthritis," *British Med. Bull.* 51:346-58; Marchalonis et al. (1994), "Synthetic Autoantigens of Immunoglobulins and T-Cell Receptors: Their Recognition in Aging, Infection, and Autoimmunity," *Autoantibodies Immunoglobulins T Cell Receptors,* pp. 129-47; Marchalonis et al. (1993), "Natural Human Antibodies to Synthetic Peptide Autoantigens: Correlations with Age and Autoimmune Disease," *Gerontology*, 39:65-79; Marchalonis et al. (1992), "Human Autoantibodies Reactive with Synthetic Autoantigens from T-Cell Receptor β Chain," *Proc. Natl. Acad. Sci. USA* 89:3325-29; "Sakkas, et al. (1994), "T-Cell Antigen Receptors in Rheumatoid Arthritis," *Immunol. Res.* 13:117-38; and Theofilpooulos et al. (1989), "B and T Cell Antigen Repertoires in Lupus/Arthritis Murine Models," *Springer Semin Immunopathol.* 11:335-68; Cronstein et al. (1994) *Curr. Opin. Rheum.* 6:300-304; Szekanecz et al. (1996) *J. Invest. Med.* 44:124-135; Liao et al. (1995) *Rheum. Arth.* 21:715-740; Veale et al. (1996) *Drugs & Aging* 9:87-92; Cronstein et al. (1994) *Curr. Opin. Rheum.* 6:300-304; Haskard (1995) *Curr. Opin. Rheum.* 7:229-234: Cronstein et al. (1994) *Curr. Opin. Rheum.* 6:300-304; Remy et al. (1995) *Proc. Natl. Acad. Sci. USA* 92:1744-1748; Ashkenas et al. (1996) *Dev. Biol.* 180:433-444; Springer (1994) *Cell* 76:301-314; Hynes (1992) *Cell* 69:11-25; and Schwartz et al. (1995) *Annu. Rev. Cell Dev. Biol.* 11:549-599.

Additional targeting ligands which may be employed in the compositions and methods of the present invention are described in Schwarzenberger et al. (1996), "Targeting Gene Transfer to Human Hematopoietic Progenitor Cell Lines Through the c-kit Receptor," *Blood.* 87:472-8; Prokopova et al. (1993), "Methyl-α-D-Mannopyranoside, Monooligosaccharides and Yeast Mannans Inhibit Development of Rat Adjuvant Arthritis," *J. Rheumatol.* 20:673-7; U.S. Patent No. 5,627,263; ," Chen, et al. (1987), "The Platelt Glycoprotein IIb/IIIa-Like Protein in Human Endothelial Cells Promotes Adhesion but not Initial Attachment to Extracellular Matrix," *J. Cell. Biol.* 105:1885-92; and Wallner et al. (1987), "Primary Structure of Lymphocyte Function-Associated Antigen 3 (LFA-3)," *J. Exp. Med.* 166:923-32.

Still additional targeting ligands that may be employed in the compositions and methods of the present invention are described in PCT publication WO 96/37194, and include fetuin and asialofetuin, hexamine, spermine and spermidine, N–glutaryl DOPE, IgA, IgM, IgG and IgD, MHC and HLA markers, and CD1, CD4, CD8–11, CD15, Cdw17, CD18, CD21-25, CD27, CD30-45, CD46-48, Cdw49, Cdw50, CD51, CD53-54, Cdw60, CD61-64, Cdw65, CD66-69, Cdw70, CD71, CD73-74, Cdw75, CD76-77, LAMP-1 and LAMP-2.

Exemplary covalent bonds through which the targeting ligands may be covalently linked to the termini of the outer arms include, for example: amide (-CONH-); thioamide (-CSNH-); ether (ROR', where R and R' may be the same or different and are other than hydrogen); ester (-COO-); thioesters (-COS-); -O-; -S-; -Sn-, where n is greater than 1, preferably about 2 to about 8, and more preferably about 2; carbamates; -NH-; -NR-, where R is alkyl, for example, alkyl of from 1 to about 4 carbons; urethane; substituted imidate; and combinations of two or more of these. Covalent bonds between targeting ligands and stabilizing materials, for example, lipids, may be achieved through the use of molecules that may act as spacers to increase the conformational and topographical flexibility of the ligand. Examples of such spacers include, for example, succinic acid, 1,6-hexanedioic acid, 1,8-octanedioic acid, and the like, as well as modified amino acids, such as, for example, 6-aminohexanoic acid, 4-aminobutanoic acid, and the like. In addition, in the case of targeting ligands that comprise peptide moieties, sidechain-to-sidechain crosslinking may be complemented with sidechain-to-end crosslinking and/or end-to-end crosslinking. Also, small spacer molecules, such as dimethylsuberimidate, may be used to accomplish similar objectives. The use of agents, including those used in Schiff's base-type reactions, such as gluteraldehyde, may also be employed. The Schiff's base linkages, which may be reversible linkages, can be rendered more permanent covalent linkages via the use of reductive amination procedures. This may involve, for example, chemical reducing agents, such as lithium aluminum hydride reducing agents or their milder analogs, including lithium aluminum diisobutyl hydride (DIBAL), sodium borohydride ($NaBH_4$) or sodium cyanoborohydride ($NaBH_3CN$).

The covalent linking of the targeting ligands to the stabilizing materials in the present compositions may be accomplished using synthetic organic techniques that would be readily apparent to one of ordinary skill in the art in view of the present disclosure. For

example, the targeting ligands may be linked to the materials via the use of well-known coupling or activation agents. As known to the skilled artisan, activating agents are generally electrophilic, which can be employed to elicit the formation of a covalent bond. Exemplary activating agents that may be used include, for example, carbonyldiimidazole (CDI), dicyclohexylcarbodiimide (DCC), diisopropylcarbodiimide (DIC), methyl sulfonyl chloride, Castro's Reagent, and diphenyl phosphoryl chloride.

The covalent bonds may involve crosslinking and/or polymerization. Crosslinking preferably refers to the attachment of two chains of polymer molecules by bridges, composed of an element, a group, or a compound, which join certain carbon atoms of the chains by covalent chemical bonds. For example, crosslinking may occur in polypeptides that are joined by the disulfide bonds of the cysteine residue. Crosslinking may be achieved by any number of methods including the addition of a chemical substance (crosslinking agent) and exposing the mixture to heat, and the exposure of the polymer to high energy radiation. A variety of crosslinking agents, or "tethers", of different lengths and/or functionalities are described, for example, in R.L. Lunbland (1995) *Techniques in Protein Modification*, CRC Press, Inc., Ann Arbor, MI, pp. 249-68. Exemplary crosslinkers include, for example, 3,3'-dithiobis(succinimidylpropionate), dimethyl suberimidate, and its variations thereof, based on hydrocarbon length, and bis-N-maleimido-1,8-octane.

In accordance with preferred embodiments, the targeting ligands may be linked or attached via a linking group. Preferably, the targeting ligand is attached via a linker that is also attached to the arms of the polymer. A variety of linking groups are available and would be apparent to one skilled in the art in view of the present disclosure. Preferably, the linking group comprises a hydrophilic polymer. Suitable hydrophilic linker polymers include, for example, polyalkyleneoxides such as, for example, PEG and polypropylene glycol (PPG), polyvinylpyrrolidones, polyvinylmethylethers, polyacrylamides, such as, for example, polymethacrylamides, polydimethylacrylamides and polyhydroxypropylmethacrylamides, polyhydroxyethyl acrylates, polyhydroxypropyl methacrylates, polymethyloxazolines, polyethyloxazolines, polyhydroxyethyloxazolines, polyhyhydroxypropyloxazolines, polyvinyl alcohols, polyphosphazenes, poly(hydroxyalkylcarboxylic acids), polyoxazolidines, polyaspartamide, and polymers of sialic acid (polysialics). The hydrophilic polymers are preferably selected from the group consisting of PEG, PPG, polyvinylalcohol and

polyvinylpyrrolidone and copolymers thereof, with PEG and PPG polymers being more preferred and PEG polymers being even more preferred. Preferred among the PEG polymers are, for example, bifunctional PEG having a molecular weight of about 1,000 Daltons to about 10,000 Daltons, preferably about 5,000 Daltons. Preferably, the polymer is bifunctional with the targeting ligand bound to a terminus of the polymer. Generally, the targeting ligand may be incorporated into the stabilizing agent at concentrations of from about 0.1 mole % to about 25 mole %, preferably from about 1 mole % to about 10 mole %. Of course, the particular ratio employed may depend upon the particular targeting ligand, linker group, and stabilizing agents.

Standard peptide methodology may be used to link the targeting ligand to the stabilizing materials when utilizing linker groups having two unique terminal functional groups. Bifunctional hydrophilic polymers, and especially bifunctional PEGs, may be synthesized using standard organic synthetic methodologies. In addition, many of these materials are available commercially, such as, for example, α-amino-ϖ-carboxy-PEG which is commercially available from Shearwater Polymers (Huntsville, AL). An advantage of using a PEG material as the linking group is that the size of the PEG may be varied such that the number of monomeric subunits of ethylene glycol may be as few as about 5, or as many as about 500 or even greater. Accordingly, the "tether" or length of the linkage may be varied, as desired. This may be important depending on the particular targeting ligand employed. For example, a targeting ligand that comprises a large protein molecule may require a short tether, and thereby simulate a membrane-bound protein. A short tether may also allow for a delivery polymer to maintain a close proximity to the target. This may be used advantageously in connection with vesicles that also comprise a bioactive agent in that the concentration of bioactive agent that may be delivered to the cell may be advantageously increased. Another suitable linking group that may provide a short tether is glyceraldehyde. Glyceraldehyde may be bound to DPPE via a Schiff's base reaction. Subsequent Amadori rearrangement can provide a substantially short linking group. The gamma carbonyl of the Schiff's base may then react with a lysine or arginine of the targeting protein or peptide to form the targeted lipid.

In certain embodiments, the targeting ligands may be incorporated in the present polymers via non-covalent associations. As known to those skilled in the art, non-covalent

association is generally a function of a variety of factors, including, for example, the polarity of the involved molecules, the charge (positive or negative), if any, of the involved molecules, the extent of hydrogen bonding through the molecular network, and the like. Non-covalent bonds are preferably selected from the group consisting of ionic interaction, dipole-dipole interaction, hydrogen bonds, hydrophilic interactions, van der Waal's forces, and any combinations thereof. Non-covalent interactions may be employed to bind the targeting ligand to the stabilizing agent. Additional techniques that may be adapted for incorporating the targeting ligand into the present compositions are disclosed, for example, in U.S. Application Serial No. 09/218,660, filed December 28, 1998.

C. The Active Agent

The drug in the formulation, as noted above, is any active agent whose systemic bioavailability can be enhanced by increasing the solubility of the agent in water. Generally, such drugs will be at least about one-and-one-half times as soluble in the hydrophilic polymer as in water, and preferably at least about ten times as soluble in the hydrophilic polymer as in water. The latter group of drugs is generally "hydrophobic" as defined in section (A). Any number of drugs may be incorporated into the formulations of the invention, i.e., any compounds that fit the aforementioned solubility criteria and induce a desired systemic effect. Such substances include the broad classes of compounds normally administered systemically. In general, this includes: analgesic agents; antiarthritic agents; respiratory drugs, including antiasthmatic agents and drugs for preventing reactive airway disease; antibiotics; anticancer agents, including antineoplastic drugs; anticholinergics; anticonvulsants; antidepressants; antidiabetic agents; antidiarrheals; antihelminthics; antihistamines; antihyperlipidemic agents; antihypertensive agents; antiinflammatory agents; antimetabolic agents; antimigraine preparations; antinauseants; antiparkinsonism drugs; antipruritics; antipsychotics; antipyretics; antispasmodics; antiviral agents; anxiolytics; attention deficit disorder (ADD) and attention deficit hyperactivity disorder (ADHD) drugs; cardiovascular preparations including cardioprotective agents; central nervous system stimulants; cough and cold preparations, including decongestants; diuretics; genetic materials; gonadotropin releasing hormone (GnRH) inhibitors; herbal remedies; hormonolytics; hypnotics; immunosuppressive agents; leukotriene inhibitors; mitotic

inhibitors; muscle relaxants; parasympatholytics; peptide drugs; psychostimulants; sedatives; steroids; sympathomimetics; tranquilizers; vasodilators, including peripheral vascular dilators; and vitamins.

It will be appreciated that the invention is particularly useful for delivering active agents for which chronic administration may be required, as the present formulations provide for sustained release. The invention is thus advantageous insofar as patient compliance with regard to forgotten or mistimed dosages is substantially improved. Any agent that is typically incorporated into a capsule, tablet, troche, liquid, suspension or emulsion, wherein administration is on a regular (i.e., daily, more than once daily, every other day, or any other regular schedule) can be advantageously delivered using the formulations of the invention.

Examples of drugs for which a sustained release formulation is particularly desirable include, but are not limited to, the following:

analgesic agents--hydrocodone, hydromorphone, levorphanol, oxycodone, oxymorphone, codeine, morphine, alfentanil, fentanyl, meperidine and sufentanil, diphenylheptanes such as levomethadyl, methadone and propoxyphene, and anilidopiperidines such as remifentanil;

ntiandrogens--bicalutamide, flutamide, hydroxyflutamide, zanoterine and nilutamide;

anxiolytic agents and tranquilizers--diazepam, alprazolam, chlordiazepoxide, clonazepam, halazepam, lorazepam, oxazepam and clorazepate;

antiarthritic agents--hydroxychloroquine, gold-based compounds such as auranofin, aurothioglucose and gold thiomalate, and COX-2 inhibitors such as celecoxib and rofecoxib;

antibiotics (including antineoplastic antibiotics)--vancomycin, bleomycin, pentostatin, mitoxantrone, mitomycin, dactinomycin, plicamycin and amikacin;

anticancer agents, including antineoplastic agents--paclitaxel, docetaxel, camptothecin and its analogues and derivatives (e.g., 9-aminocamptothecin, 9-nitrocamptothecin, 10-hydroxy-camptothecin, irinotecan, topotecan, 20-O-β-glucopyranosyl camptothecin), taxanes (baccatins, cephalomannine and their derivatives), carboplatin, cisplatin, interferon-α_{2A}, interferon-α_{2B}, interferon-α_{N3} and other agents of the interferon family, levamisole, altretamine, cladribine, bovine-calmette-guerin (BCG), aldesleukin, tretinoin, procarbazine, dacarbazine, gemcitabine, mitotane, asparaginase, porfimer, mesna,

amifostine, mitotic inhibitors including podophyllotoxin derivatives such as teniposide and etoposide and vinca alkaloids such as vinorelbine, vincristine and vinblastine;

antidepressant drugs--selective serotonin reuptake inhibitors such as sertraline, paroxetine, fluoxetine, fluvoxamine, citalopram, venlafaxine and nefazodone; tricyclic anti-depressants such as amitriptyline, doxepin, nortriptyline, imipramine, trimipramine, amoxapine, desipramine, protriptyline, clomipramine, mirtazapine and maprotiline; other anti-depressants such as trazodone, buspirone and bupropion;

antiestrogens--tamoxifen, clomiphene and raloxifene;

antifungals—amphotericin B, imidazoles, triazoles, and griesofulvin;

antihyperlipidemic agents--HMG-CoA reductase inhibitors such as atorastatin, simvastatin, pravastatin, lovastatin and cerivastatin sodium, and other lipid-lowering agents such as clofibrate, fenofibrate, gemfibrozil and tacrine;

antimetabolic agents--methotrexate, fluorouracil, floxuridine, cytarabine, mercaptopurine and fludarabine phosphate;

antimigraine preparations--zolmitriptan, naratriptan, sumatriptan, rizatriptan, methysergide, ergot alkaloids and isometheptene;

antipsychotic agents--chlorpromazine, prochlorperazine, trifluoperazine, promethazine, promazine, thioridazine, mesoridazine, perphenazine, acetophenazine, clozapine, fluphenazine, chlorprothixene, thiothixene, haloperidol, droperidol, molindone, loxapine, risperidone, pimozide and domepezil;

aromatase inhibitors--anastrozole and letrozole;

attention deficit disorder and attention deficit hyperactivity disorder drugs--methylphenidate and pemoline;

cardiovascular preparations--angiotensin converting enzyme (ACE) inhibitors; diuretics; pre- and afterload reducers; cardiac glycosides such as digoxin and digitoxin; inotropes such as amrinone and milrinone; calcium channel blockers such as verapamil, nifedipine, nicardipene, felodipine, isradipine, nimodipine, bepridil, amlodipine and diltiazem; beta-blockers such as pindolol, propafenone, propranolol, esmolol, sotalol and acebutolol; antiarrhythmics such as moricizine, ibutilide, procainamide, quinidine, disopyramide, lidocaine, phenytoin, tocainide, mexiletine, flecainide, encainide, bretylium and amiodarone; cardioprotective agents such as dexrazoxane and leucovorin;

GnRH inhibitors and other hormonolytics and hormones--leuprolide, goserelin, chlorotrianisene, dinestrol and diethylstilbestrol;

herbal remedies--melatonin;

immunosuppressive agents--6-thioguanine, 6-aza-guanine, azathiopurine, cyclosporin and methotrexate;

lipid-soluble vitamins--tocopherols and retinols;

leukotriene inhibitors--zafirlukast, zileuton and montelukast sodium;

nonsteroidal anti-inflammatory drugs (NSAIDs)--diclofenac, flurbiprofen, ibuprofen, ketoprofen, piroxicam, naproxen, indomethacin, sulindac, tolmetin, meclofenamate, mefenamic acid, etodolac, ketorolac and bromfenac;

peptide drugs--leuprolide, somatostatin, oxytocin, calcitonin and insulin;

peripheral vascular dilator--cyclandelate, isoxsuprine and papaverine;

respiratory drugs--such as theophylline, oxytriphylline, aminophylline and other xanthine derivatives;

steroids--progestogens such as flurogestone acetate, hydroxyprogesterone, hydroxyprogesterone acetate, hydroxyprogesterone caproate, medroxyprogesterone acetate, megestrol, norethindrone, norethindrone acetate, norethisterone, norethynodrel, desogestrel, 3-keto desogestrel, gestadene and levonorgestrel; estrogens such as estradiol and its esters (e.g., estradiol benzoate, valerate, cyprionate, decanoate and acetate), ethynyl estradiol, estriol, estrone, mestranol and polyestradiol phosphate; corticosteroids such as betamethasone, betamethasone acetate, cortisone, hydrocortisone, hydrocortisone acetate, corticosterone, fluocinolone acetonide, flunisolide, fluticasone, prednisolone, prednisone and triamcinolone; androgens and anabolic agents such as aldosterone, androsterone, testosterone and methyl testosterone; and

topoimerase inhibitors—camptothecin, anthraquinones, anthracyclines, teniposide, etoposide, topotecan and irinotecan.

Genetic material may also be delivered using the present formulation, e.g., a nucleic acid, RNA, DNA, recombinant RNA, recombinant DNA, antisense RNA, antisense DNA, hammerhead RNA, a ribozyme, a hammerhead ribozyme, an antigene nucleic acid, a ribooligonucleotide, a deoxyribonucleotide, an antisense ribooligonucleotide, and an antisense deoxyribooligonucleotide. Representative genes include vascular endothelial

growth factor, fibroblast growth factor, BCl-2, cystic fibrosis transmembrane regulator, nerve growth factor, human growth factor, erythropoeitin, tumor necrosis factor, interleukin-2 and histocompatibility genes such as HLA-B7.

The foregoing list is merely illustrative and is not intended to be limiting. A wide variety of drugs and drug types can be effectively administered using the present formulations, although the invention is most advantageous with hydrophobic drugs.

It may be desirable to include one or more P-glycoprotein inhibitors in the formulation along with the active agent to be administered. It has been established that intestinal absorption of certain drugs, of which paclitaxel is exemplary, is controlled by P-glycoprotein (P-gp). With such drugs, then, the present formulations preferably include a P-gp inhibitor for oral administration in order to increase intestinal absorption and thus oral bioavailability. A particularly preferred P-gp inhibitor is cyclosporin A, although other P-gp inhibitors may also be used. When a P-gp inhibitor is included in the formulation, the weight ratio of drug to P-gp inhibitor (e.g., the ratio of paclitaxel to cyclosporin A) will generally be in the range of about 1:5 to 5:1, preferably in the range of about 1:2 to 2:1, more preferably in the range of about 1:1.5 to 1.5:1, and optimally about 1:1. With paclitaxel, it may also be desirable to co-administer a folate (i.e., a salt or ester of folic acid), which has been found to increase paclitaxel absorption.

The amount of drug in the formulation should be such that the weight ratio of drug to all other components of the formulation is in the range of about 1:1 to 1:50, preferably in the range of about 1:1 to 1:20, more preferably in the range of about 1:2 to 1:10, and optimally about 1:5.

D. Other Components of the Formulation

Free phospholipids, i.e., phospholipids not conjugated to PEG or other moieties, may be incorporated into the present formulations as excipients in order to reduce the particle size of the polymer/drug matrix. For intravenous administration in particular, particle size is critical, and is generally in the range of about 1 nm to 10 μm, preferably in the range of about 5 nm to 500 nm, most preferably in the range of about 30 nm to 250 nm (the values given are number average). The free phospholipid, like the phospholipid that may be conjugated to the hydrophilic polymer, can be anionic, neutral or cationic, of naturally occurring or synthetic

origin, and will generally comprise a diacyl phosphatidylcholine, a diacyl phosphatidylethanolamine, a diacyl phosphatidylserine, a diacyl phosphatidylinositol, a diacyl phosphatidylglycerol or a diacyl phosphatidic acid, wherein each acyl moiety can be saturated or unsaturated and typically contains about 8 to 20 carbon atoms. As with the conjugated phospholipids, the preferred unsaturated acyl moieties of the free phospholipids are oleic and linoleic acid esters, and preferred saturated acyl moieties are palmitate, myristate and stearate; particularly preferred phospholipids are DPPE and POPE. The amount of free phospholipid should be just sufficient to reduce the particle size as desired. Preferably, any free phospholipid that is included in the formulation represents less than about 25% of the total phospholipid present, and optimally represents less than about 10% of the total phospholipid present.

Stabilizing agents may also be added to the formulation and are useful for reducing particle size. The polymers may be used in addition or in lieu of free phospholipids. Preferably, the stabilizing agent acts to stabilize the surface of the complex by virtue of a combination of hydrophilic and hydrophobic interactions. Thus, it is preferred that the stabilizing agentpolymer contains both hydrophilic and hydrophobic groups or domains thus allowing this interaction to occur. It is also preferred that the stabilizing agent contain a sufficient amount of hydrophilic surfaces that post stabilization nanoparticles remain suspended within water and avoid clumping.

The preferred stabilizing agent is a polymer having a molecular weight ranging from about 400 Daltons to about 400,000 Daltons, more preferably from about 1,000 Daltons to about 200,000 Daltons, and still more preferably from about 3,000 Daltons to about 100,000 Daltons. The stabilizing agent may be derived from natural, recombinant, synthetic or semisynthetic sources. Most preferably the stabilizing agent will be a protein or a peptide. Useful preferred proteins include albumin, collagen, fribrin, immunoglobulins, hemoglobin, vascular endothelial growth factor, vascular permeability factor, epidermal growth factor, fibroblast growth factor, fibronectin, vitronectin, and cytokines such as interleukins (e.g. IL-3 and IL-12).

Suitable stabilizing proteins include, but are not limited to: serum proteins, i.e., albumin (especially recombinant and defatted), arnylins, atrial natriuretic peptides, endothelins and endothelin inhibitors, urokinase, streptokinase, staphylokiase, vasoactive

intestinal peptide, HDL, LDL, VLDL, etc.; agglutination (antihemophillia) factors, i.e., Factor VIII, Factor IX and subtypes thereof, decorsin, serum thymic factor, etc.; peptide hormones, i.e., ACTH, FSH, LH, parathyroid hormone, thyroxin, insulin, vasopressin, bradykinin and bradykinin potentiators, HGH, CRF (corticotropin releasing factor), oxytocin, gastrins, LH-RH, MSH (melanocyte stimulating hormone) and MSH releasing factor; parathyroid hormones and analogs; pituitary adenylate cyclase activating polypeptide; secretins; thyrotropin releasing hormone, etc.; structural proteins, i.e., collagens, amyloid proteins, brain natriuretic peptides, elafin, fibronectin and fibronectin fragments, laminin, sarafotoxins, etc.; growth factors, i.e., nerve growth factor, platelet derived growth factor, epidermal growth factor, vascular endothelial growth factor, tumor necrosis factor, CINC-I (cytokine-induced neutrophil chemoattractant), growth hormone releasing factor, liver cell growth factor, midkines, neurokinins, neuromedins, etc., metabolic potentiators, i.e., erythropoietin, adrenomedullin and adrenomedullin antagonists, (o-agatoxin TK, agelenin, angiotensins, calcicludine, calciseptine, calcitonin and calcitonin antagonists, calmodulin, charybdotoxin, chlorotoxin, conotoxins, endorphins, neo-endorphins, glucagon and variants, guanylins, iberiotoxin, kaliotoxin, margatoxin, mast cell degranulating peptide, neurotensins, pancreastatins, PLTX-11, scylotoxin, ATPase inhibitors, somatostatins, somatomedin, uroguanylin, etc.; nuclear binding proteins, i.e., histones, spermine, spermidine, nuclear localization sequences, telomerase, etc.; enzymes, i.e., cholecystokinin, cathepsins, etc.; antivirals, i.e., IFN-oc, IFN-P, IFN-Z, virus replication inhibiting peptide, etc.; immunoglobulins, i.e., IgA, IgD, IgE, IgG, IgH and subtypes; and miscellaneous proteins such as apamin, bombesin, casomorphins, conantokins, defensin-1, dynorphins, enkephalins, galanins, magainin, nociceptin, osteocalcins, substance P, xenin, etc. While not wishing to be limited to the preceding examples, one of skill in the art will recognize that the examples given may be used individually or in combination.

The stabilizing protein may also serve as a targeting agent or binding ligand to direct the nanoparticles and drugs therein to a certain site. The preferred protein is albumin, in particular, human serum albumin and even more preferably recombinant derived human albumin. Another preferred protein is defatted albumin, either native or recombinant. For veterinary applications, the albumin is preferably from the patient's species. The stabilizing albumin is generally added to the nanoparticles at an effective stabilizing concentration,

generally in the range of about 0.001% w/v to up to about 10% w/v, more preferably in the range of about 0.01% to about 5%, in the range of about 0.1 % to about 2.5%, and most preferably in the range of about 0.25% to about 1.5%. Note that more than one protein may be used to stabilize the nanoparticles. For example, the particles may be formulated with about 1.0% w/v albumin and about 0.1% w/v EGF. In this case, the EGF serves as a targeting ligand to help the nanoparticle to bind to tissues with increased expression of the EGF receptor.

The protein may be naturally occurring, a protein fragment, e.g. a fragment of the gamma-carboxy terminus of fibrinogen, or chemically modified. For example, albumin or other proteins may be modified with one or more hydrophilic or targeting moieties. For example, the protein may be modified by binding one or more PEG residues per protein molecule, typically between 1 and 100 PEG molecules per protein molecule, but more preferably between 1 and 10 PEG residues. For example, mono or bifunctional PEG groups may be coupled to the protein through linkages such as ethers or biodegradable bonds such as esters, amides, carbamates, thioesters, disulfides, thiocarbamates, phosphate esters and phosphoamides. The resulting "PEGylated" protein enables the protein to stabilize the surface of the nanoparticle while the PEG groups help to protect the nanoparticle surface from nonspecific interaction with serum proteins. In this regard, the "PEGylated" proteins increase the serum half-lives of the nanoparticles.

In addition to the proteins enumerated above, the polymers may be other natural polymers, such as: cellulose and dextran; semi-synthetic cellulose derivatives such as methylcellulose and carboxymethylcellulose; and synthetic polymers such as polvinylalcohol polyvinylpyrrolidone and copolymers containing PEG and a second polymer such as polypropylene glycol (PPG) (e.g. those available under the Pluronic trademark). Synthetic polymers such as the Pluronics, i.e. copolymers of PEG and PPG, may be incorporated into mixtures of stabilizing agents, e.g., with albumin. Preferred block copolymers include, but are not limited to, polyethylene glycol-N-carboxyanhydride of 6-(benzyloxycarbonyl)-l-lysine, polyethylene glycol-poly-l-lysine and polyethylene glycol-polyaspartic acid. Methods for synthesizing the above copolymers are detailed in Harada and Kataoka (1995) *Macromolecules* 28:5294-5299. One of skill in the art will readily recognize that the same synthetic methods can be used to substitute polypropylene glycols for PEG to make the PPG

block copolymer analogs of the above.

Other suitable PEG copolymers may be synthesized from polymerizable aldehydes that optionally contain additives and/or crosslinking elements capable of copolymerization, surfactants or surfactant mixtures, coupling agents, biomolecules or macromolecules bound by these coupling agents, as well as diagnostically or therapeutically effective components.

The monomers encompassed herein include, but are not limited to, alpha/beta-unsaturated aldehydes, e.g., acrolein, crotonaldehyde, propionaldehyde, alpha-substituted acrolein derivatives, e.g., alpha-methyl acrolein, alpha-chloroacrolein, alpha-phenyl acrolein, alpha-ethyl acrolein, alpha-isopropyl acrolein, alpha-n-butyl acrolein, alpha-n-propyl acrolen; dialdehydes, e.g., glutaraldehyde, succinaldehyde or their derivatives or their mixtures with additives capable of copolymerization (comonomers), e.g., alpha-substituted acroleins, beta-substituted acroleins, ethyl cyanoacrylates, methyl cyanoacrylates, butyl cyanoacrylates, hexyl eyanoacrylate, methyl methacrylates, vinyl alcohols, acrylic acids, methacrylic acids, acrylic acid chlorides, methacrylic acid chlorides, acrylonitrile, methacrylonitriles, acrylarnides, substituted acrylamides, hydroxy methyl methacrylates, mesityl oxide, dimethylaminoethylmethacrylates 2-vinylpyridines and N-vinyl-2-pyrrolidinone.

Suitable coupling agents that may be employed in the synthesis of PEG copolymers include, but are not limited to: compounds containing amino groups (e.g., hydroxylamine, butylamine, allylamine, ethanolamine, trishydroxymethylaminomethane, 3-amino-1-propanesulfonic acid, 5-aminovaleric acid, 8-aminooetanoic acid, D-glucosamine hydrochloride, aminogalactose, aminosorbitol, aminomannitol, diethylaminoethylamine, anilines, sulfonyl acid amide, choline, N-methylglucamine, piperazine, 1,6-hexanediamine, urea, hydrazine, glycine, alanine, lysine, serine, valine, leucine, peptides, proteins, albumin, human serum albumin, polylysine, gelatin, polyglycolamines, aminopolyalcohols, dextran sulfates with amino groups, N-aminopolyethylene glycol ($HO\text{-}PEG\text{-}NH_2$), N,N'-diaminopolyethylene glycol ($NH_2\text{-}PEG\text{-}NH_2$), antibodies, immunoglobulins, etc.); compounds containing acid groups, e.g., carboxylic acids such as acetic acid, propionic acid, butyric acid, valeric acid, caproic acid, caprylic acid, capric acid, lauric acid, myristic acid, palmitic acid, stearic acid, oleic acid, linolic acid, linolenic acid, cyclohexane carboxylic acid, phenylacetic acid, benzoylacetic acid, chlorobenzoic acid, bromobenzoic acid, nitrobenzoic acid, ortho-phthalic acid, meta-phthalic acid, para-phthalic acid, salicylic acid,

hydroxybenzoic acid, aminobenzoic acid, methoxybenzoic acid, PEG-linker-glutaminic acid, PEG-linker-DTPA, PEG-linker-EDTA, etc.; compounds containing hydroxy groups, i.e., alcohols such as methanol, ethanol, propanol, butanol, pentanol, hexanol, heptanol, octanol, decanol, dodecanol, tetradecanol, hexadecanol, octadecanol, isopropyl alcohol, isobutyl alcohol, isopentyl alcohol, cyclopentanol, cyclohexanol, crotyl alcohol, benzyl alcohol, phenyl alcohol, diphenyl methanol, triphenyl methanol, cinnamyl alcohol, ethylene glygol, 1,3-propanediol, glycerol, pentaerythritol and the like; polymerizable substances, such as alpha, beta-unsaturated aldehydes, e.g., acrolein, crotonaldehyde, propionaldehyde, etc.; alpha-substituted acrolein derivatives, e.g., alpha-methylacrolein, alpha-chloroacrolein, alpha-phenylacrolein, alpha-ethylacrolein, alpha-isopropylacrolein, alpha-n-butylacrolein, alpha-n-propylacrolein, etc.; and dialdehydes, e.g., glutaraldehyde, succinaldehyde or their derivatives or their mixtures with additives capable of copolymerization, such as alpha-substituted acroleins, beta-substituted acroleins, ethyl cyanoacrylates, methyl cyanoacrylates, butyl acrylates, hexyl cyanoacrylates, methylmethacrylates, vinyl alcohols, acrylic acids, methacrylic acids, acrylic acid chlorides, acrylonitrile, methacrylonitriles, acrylamides, substituted acrylamides, hydroxymethylmethacrylates, mesityl oxide, dimethylaminoethylmethacrylates 2-vinylpyridines, vinylpyrrolidinone, etc.

Particularly preferred coupling agents include hydroxylamine, trishydroxymethylaminomethane, 3-amino-1-propane sulfonic acid, D-glucosaminohydrochloride, aminomannitol, urea, human serum albumin, hydrazine, proteins, polyglycolamines, aminopolyalcohols (e.g., HO-PEG-NH_2 or NH_2-PEG-NH_2), and compounds containing acid groups such as PEG-linker-asparaginic acid, PEG-linker-glutaminic acid, PEG-linker-DTPA and PEG-linker-EDTA, wherein the molecular weight of the PEG is less than about 100 kD, preferably less than about 40 kD.

The amount of coupling agent is typically present in the range of about 1% wt. to about 95% wt. of the polyaldehyde in the PEG copolymer. The coupling agents can be condensed by their amino group or on the formyl groups located on the surface of nanoparticles synthesized from polymerized aldehydes and optional surfactants. Also, such formyl groups may also bind those monomers listed above that are polymerizable. However, the acids and alcohols named above are typically coupled on the nanoparticles only after previous conventional conversion of the aldehyde function.

Generally the stabilizing agent is added to the complex in aqueous media. The complex and stabilizing agent are then subjected to a mechanical dispersal process that helps to break the complex into nanoparticles that are then stabilized by the stabilizing agent. Useful mechanical dispersal processes include shaking, agitation (e.g., vortexing), sonication, extrusion under pressure, microfluidization, microemulsification and high speed blending.

Compounds other than free phospholipids are also useful for reducing particle size, and can be used in addition to or in lieu of free phospholipids; these other compounds include, but are not limited to, cholic acids, cholic acid salts, saccharides (such as sorbitol, sucrose and trehalose), polyhydroxyalcohols (such as glycerol), and liquid polyethylene glycols (i.e., PEG having a molecular weight less than about 1000 Daltons). The formulations of the invention can also contain pharmaceutically acceptable auxiliary agents as required in order to approximate physiological conditions; such auxiliary agents include pH adjusting and buffering agents, tonicity adjusting agents, and the like. Lipid-protecting agents that serve to minimize free radical and peroxidative damage upon storage may also be advantageous. Suitable lipid protective agents include alpha-tocopherol and water-soluble, iron-specific chelators such as deferoxamine and ethylenediaminetetraacetic acid (EDTA). Additionally, for lyophilized compositions that are to be hydrated prior to use, it may be desirable to include one or more cryoprotectants, or anti-flocculants in order to facilitate rehydration and formation of a substantially homogeneous suspension. For compositions that are to be stored in liquid form, it is preferred that one or more conventional anti-bacterial agents be included. Still other additives that may be incorporated into the present formulations include radioactive or fluorescent markers useful for imaging purposes. Radioactive markers include, for example, technitium and indium, while an exemplary fluorescent marker is fluorescein. The excipients can be included in an amount up to about 50 wt.% of the formulation, but preferably represent less than about 10 wt.% of the formulation.

E. Manufacture and Storage

The formulations of the invention are manufactured using standard techniques and reagents known to those skilled in the art of pharmaceutical formulation and drug delivery and/or described in the pertinent texts and literature. See *Remington: The Science and*

Practice of Pharmacy, 19th Ed. (Easton, PA: Mack Publishing Co., 1995), which discloses conventional methods of preparing pharmaceutical compositions that may be used as described or modified to prepare pharmaceutical formulations of the invention. In a preferred embodiment, the hydrophilic polymer, drug, optional free phospholipid and all other components of the final composition are mixed together in an organic solvent or solvent system such as isopropanol, t-butanol, benzene/methanol, ethanol, or an alternative suitable solvent as will be apparent to those of skill in the art and then lyophilizing the mixture. The solvent may also be removed by subjecting the mixture to rotary evaporation to yield a powder or a solid matrix. When a solid matrix is obtained, the material may be ground via ball milling or subjected to other mechanical shear stress to achieve a finely ground powder of nanoparticulate material. The resulting nanoparticles may be stabilized with surfactants, phospholipids, stabilizing agents including albumin, and other stabilizing materials, as discussed above. Another method of manufacturing the formulation is spray drying. In this method, a suitable organic solvent, ideally having a flash point sufficiently above the drying temperature. Formulations made using this method are in the form of a fluffy, dry powder.

Alternatively, the components of the final product may be dissolved in a supercritical fluid such as compressed carbon dioxide, and then ejected under pressure and shearing force to form dried particles of the drug-containing formulation. The formulation is preferably stored in lyophilized form, in which case, the lyophilized composition is rehydrated prior to use. Rehydration is carried out by mixing the lyophilized composition with an aqueous liquid (e.g., water, isotonic saline solution, phosphate buffer, etc.) to provide a total solute concentration in the range of about 50 to 100 mg/ml and a drug concentration in the range of about 1 to 20 mg/ml, preferably about 5 to 15 mg/ml. The formulation may, however, be stored in the aqueous state, e.g., in pre-filled syringes or vials. The formulation may also be stored as a liquid in a physiologically acceptable organic solvent such as ethanol, propylene glycol or glycerol, to be diluted with water prior to injection into a patient. The lyophilized and rehydrated formulations may be stored at various temperatures such as freezing conditions (below about 0°C and as low as about -40°C to -100°C), refrigerated conditions generally between about 0°C and 15°C, room temperature conditions generally between about 15°C and 28°C, or at elevated temperatures as high as about 40°C.

The particle size of individual particles within the formulation will vary, depending upon the molecular weight and concentration of the hydrophilic polymer, the amount of drug as well as its solubility profile (i.e., its solubility in water and the hydrophilic polymer), the use of stabilizing agents, and the conditions used in manufacturing. That is, as noted in the preceding section, stabilizing agents and various excipients may be used to facilitate rehydration and provide a substantially homogeneous dispersion. Additionally, mechanical processing techniques can be used to adjust particle size to the appropriate diameter for the intended application; for example, after rehydration, the formulation can be subjected to shear forces with microfluidization, sonication, extrusion, or the like. Formulations made with stabilizing agents can have a particle size on the order of about 20 nm to 100 nm. These smaller particles, by virtue of their larger accessible surface-to-volume ratio, tend to release drug quite rapidly, while larger particles, e.g., over 10 μm in diameter, will provide for far more gradual, sustained release of drug. The preferred particle size herein is in the range of about 1 nm to 500-1000 μm in diameter. For intramuscular and subcutaneous injection, particle size should be in the range of about 1 nm to 500 μm, preferably in the range of about 10 nm to 300 μm, and most preferably in the range of about 20 μm to 200 μm. For intravenous administration, as noted previously, particle size is optionally in the range of about 30 nm to 250 nm. For interstitial administration and fracture or wound packing, particle size can be up to 1000 μm, while for embolization, particle size will generally be between about 100 μm and 250 μm.

The formulation can be sterilized using heat, ionizing radiation or filtration. For drugs that are thermally stable, heat sterilization is preferable. Lower viscosity formulations can be filter sterilized, in which case the particle size should be under about 200 nm. Aseptic manufacturing conditions may be employed as well, and lyophilization is also helpful to maintain sterility and ensure long shelf-life. In addition, as noted in the preceding section, anti-bacterial agents may be included in aqueous formulations in order to prevent bacterial contamination.

F. INCORPORATION OF AN ACOUSTICALLY ACTIVE GAS

In a further embodiment of the invention, the present formulations are made with small quantities of an acoustically active gas instilled therein. In order to instill the selected gas into the present formulations, a headspace of gas (preferably an insoluble gas) is applied atop the lyophilized composition in a closed container, which is then exposed to mild agitation during rehydration. Microquantities of gas will become entrapped in the interstices of the dispersion. The presence of the acoustically active gas is useful in conjunction with ultrasound imaging, as the gas-instilled dispersion produces an echogenic contrast that allows the drug to be tracked in the body. In addition, if a sufficient quantity of gas is entrapped in the formulation, therapeutic ultrasound can allow the microstructure to unfold at the locus where the ultrasound is applied, releasing the active agent and thus enhancing targeting effectiveness. The acoustically active gas lowers the cavitation threshold, i.e., the energy required for cavitation with ultrasound. Preferably, the cavitation energy used will be under about 1.5 MegaPascals, and more preferably under about 1.0 MegaPascals. The gas also effects dB reflectivity, and a gas concentration of about 1 mg per ml of particles will generally have a reflectivity approximately 2 dB higher than that of pure water.

In general, the amount of acoustically active gas that is imbibed by the particles of the formulation is approximately equal to the void space within the particles, which can be approximated by their density. For example, particles having a density of 0.10 will imbibe about 90 vol.% gas. Lower density particles will imbibe a higher volume of gas (i.e., 95 vol.% for particles having a density of 0.05), while higher density particles will imbibe a lower volume of gas (i.e., 85 vol.% for particles having a density of 0.15). Gas may also adhere to the surface of the particles, typically up to about two times the volume of the particles. Normally, the amount of acoustically active gas that is employed is such that the gas-instilled formulation will contain at least about 5 vol.% gas, preferably about 10-15 vol.% gas.

Typical acoustically active gases are chemically inert gases having 1 to 12 carbon atoms, and particularly preferred acoustically active gases are perfluorocarbons, including saturated perfluorocarbons, unsaturated perfluorocarbons, and cyclic perfluorocarbons. The saturated perfluorocarbons, which are usually preferred, have the formula C_nF_{2n+2}, where n is from 1 to 12, preferably 2 to 10, more preferably 4 to 8, and most preferably 5. Examples

of suitable saturated perfluorocarbons are the following: tetrafluoromethane; hexafluoroethane; octafluoropropane; decafluorobutane; dodecafluoropentane; perfluorohexane; and perfluoroheptane. Saturated cyclic perfluorocarbons, which have the formula C_nF_{2n}, where n is from 3 to 8, preferably 3 to 6, may also be preferred, and include, e.g., hexafluorocyclopropane, octafluorocyclobutane, and decafluorocyclopentane. Other gases that can be used include air, nitrogen, helium, argon, xenon and other such gases.

Alternatively, a gaseous precursor can be used that is in the liquid state at room temperature and that is either (1) volatilized prior to introduction into the headspace above the lipid- and drug-containing dispersion, or (2) volatilized and instilled into a microemulsion which is then introduced into the lipid- and drug-containing dispersion. Suitable gaseous precursors are described, for example, in U.S. Patent No. 5,922,304 to Unger, and include, without limitation, hexafluoro acetone, isopropyl acetylene, allene, tetrafluoroallene, boron trifluoride, isobutane, 1,2-butadiene, 2,3-butadiene, 1,3-butadiene, 1,2,3-trichloro-2-fluoro-1,3-butadiene, 2-methyl-1,3-butadiene, hexafluoro-1,3-butadiene, butadiyne, 1-fluoro-butane, 2-methyl-butane, decafluorobutane, 1-butene, 2-butene, 2-methyl-1-butene, 3-methyl-1-butene, perfluoro-1-butene, perfluoro-2-butene, 4-phenyl-3-butene-2-one, 2-methyl-1-butene-3-yne, butyl nitrate, 1-butyne, 2-butyne, 2-chloro-1,1,1,4,4,4-hexafluorobutyne, 3-methyl-1-butyne, perfluoro-2-butyne, 2-bromobutyraldehyde, carbonyl sulfide, crotononitrile, cyclobutane, methylcyclobutane, octafluorocyclobutane, perfluorocyclobutene, 3-chlorocyclopentene, octafluorocyclopentenecyclopropane, 1,2-dimethylcyclopropane, 1,1-dimethylcyclopropane, 1,2-dimethylcyclopropane, ethylcyclopropane, methylcyclopropane, diacetylene, 3-ethyl-3-methyl diaziridine, 1,1,1-trifluorodiazoethane, dimethylamine, hexafluorodimethylamine, dimethylethylamine, bis(dimethylphosphine)amine, perfluorohexane, 2,3-dimethyl-2-norbornane, perfluorodimethylamine, dimethyloxonium chloride, 1,3-dioxolane-2-one, 4-methyl-1,1,1,2-tetrafluoroethane, 1,1,1-trifluoroethane, 1,1,2,2-tetrafluoroethane, 1,1,2-trichloro-1,2,2-trifluoroethane, 1,1-dichloroethane, 1,1-dichloro-1,2,2,2-tetrafluoroethane, 1,2-difluoroethane, 1-chloro-1,1,2,2,2-pentafluoroethane, 2-chloro-1,1-difluoroethane, 1,1-dichloro-2-fluoroethane, 1-chloro-1,1,2,2-tetrafluoroethane, 2-chloro-1,1-difluoroethane, chloroethane, chloropentafluoroethane, dichlorotrifluoroethane, fluoroethane, hexafluoroethane, nitropentafluoroethane, nitrosopentafluoroethane, perfluoroethylamine, ethyl vinyl ether, 1,1-dichloroethane, 1,1-dichloro-1,2-difluoroethane,

1,2-difluoroethane, methane, trifluoromethanesulfonylchloride, trifluoromethane-sulfonylfluoride, bromodifluoronitrosomethane, bromofluoromethane, bromochloro-fluoromethane, bromotrifluoromethane, chlorodifluoronitromethane, chlorodinitromethane, chlorofluoromethane, chlorotrifluoromethane, chlorodifluoromethane, dibromodifluoromethane, dichlorodifluoromethane, dichlorofluoromethane, difluoromethane, difluoroiodomethane, disilanomethane, fluoromethane, iodomethane, iodotrifluoromethane, nitrotrifluoromethane, nitrosotrifluoromethane, tetrafluoromethane, trichlorofluoromethane, trifluoromethane, 2-methylbutane, methyl ether, methyl isopropyl ether, methyllactate, methylnitrite, methylsulfide, methyl vinyl ether, neon, neopentane, nitrogen (N_2), nitrous oxide, 1,2,3-nonadecane-tricarboxylic acid-2-hydroxytrimethylester, 1-nonene-3-yne, oxygen (O_2), 1,4-pentadiene, n-pentane, perfluoropentane, 4-amino-4-methylpentan-2-one, 1-pentene, 2-pentene (cis), 2-pentene (trans), 3-bromopent-1-ene, perfluoropent-1-ene, tetrachlorophthalic acid, 2,3,6-trimethylpiperidine, propane, 1,1,1,2,2,3-hexafluoropropane, 1,2-epoxypropane, 2,2-difluoropropane, 2-aminopropane, 2-chloropropane, heptafluoro-1-nitropropane, heptafluoro-1-nitrosopropane, perfluoropropane, propene, hexafluoropropane, 1,1,1,2,3,3-hexafluoro-2,3 dichloropropane, 1-chloropropane, chloropropane (trans), 2-chloropropane, 3-fluoropropane, propyne, 3,3,3-trifluoropropyne, 3-fluorostyrene, sulfur hexafluoride, sulfur (di)-decafluoride (S_2F_{10}), 2,4-diaminotoluene, trifluoroacetonitrile, trifluoromethyl peroxide, trifluoromethyl sulfide, tungsten hexafluoride, vinyl acetylene, vinyl ether, and xenon.

III. UTILITY:

The formulations of the invention are used to treat a mammalian individual, generally a human patient, suffering from a condition, disease or disorder that is responsive to systemic administration of a particular drug. The formulations may be administered orally, parenterally, topically, transdermally, rectally, vaginally, by inhalation, intra-ocularly, in an implanted reservoir (i.e., in a sustained release depot for subcutaneous or intramuscular administration), or as a packing material for wounds and fractures. The term "parenteral" as used herein is intended to include subcutaneous, intravenous, intramuscular, intra-arterial, intrathecal and intraperitoneal injection, and the formulation may be injected as either a bolus or an infusion. Since the invention substantially increases the systemic bioavailability of a

drug having low aqueous solubility, dosage can be significantly reduced relative to that used in conjunction with conventional pharmaceutical compositions containing the same active agent. Analogously, a conventional dosage--or an increased dosage--can be used to provide substantially higher blood levels of a drug than previously obtained using conventional formulations. For paclitaxel, by way of example, a bolus dosage of at least 3.5 mg/kg and even 7.0 mg/kg can be administered using the present formulations, while with continuous infusion, a dosage of at least 140 mg/kg can be administered and with using a stabilized formulation, a dosage of up to 200 mg/kg can be administered.

The formulations of the invention may be also be used so that a drug is targeted to a particular cell type or tissue. In this embodiment, a targeting agent is employed that is covalently coupled to the hydrophilic polymer such as through a terminal hydroxyl group of polyethylene glycol. Suitable targeting agents are those that are generally used with liposomal formulations, e.g., peptides, peptide fragments, antibodies or peptidomimetics, although other ligands such as saccharides and folates can also be used. Preferred targeting agents are integrins such as the β_3 integrins ("cytoadhesins"), with cyclized oligopeptides containing the Arg-Gly-Asp (RGD) motif particularly preferred.

The present formulations are also useful as packing materials for wounds and fractures, and as coating materials for endoprostheses such as stents, grafts and joint prostheses. It is known that restenosis (narrowing of the blood vessels) may occur after angioplasty, placement of a stent, and/or other coronary intervention procedures, as a result of fibroblast proliferation and smooth muscle hypertrophy. Thus, the formulations of the invention may be used as coating materials for endoprostheses to provide local drug delivery following coronary intervention.

It is to be understood that while the invention has been described in conjunction with the preferred specific embodiments thereof, that the foregoing description as well as the examples that follow are intended to illustrate and not limit the scope of the invention. Other aspects, advantages and modifications within the scope of the invention will be apparent to those skilled in the art to which the invention pertains.

All patents, patent applications, and publications mentioned herein are hereby incorporated by reference in their entirety.

EXPERIMENTAL

The following examples are put forth so as to provide those of ordinary skill in the art with a complete disclosure and description of how to prepare and use the formulations disclosed and claimed herein. Efforts have been made to ensure accuracy with respect to numbers (e.g., amounts, temperature, etc.) but some errors and deviations should be accounted for. Unless indicated otherwise, parts are parts by weight, temperature is in °C and pressure is at or near atmospheric.

Also, in these examples and throughout this specification, the abbreviations employed have their generally accepted meanings, as follows:

g = gram
ml = milliliter
mmol = millimole
nm = nanometer
µl = microliter
µm = micron

EXAMPLE 1

The solubility of various drugs was evaluated in both polyethylene glycol and water. The drugs tested and their source, catalog number and lot number are given in Table 1.

TABLE 1

COMPOUND	MANUFACTURER	CATALOG #	LOT #
trans- retinol (vitamin A)	Sigma	R-7632	69H5019
α-tocopherol (vitamin E)	Sigma	T-3251	6981224
dexamethasone	Sigma	D 1756	88H1266
dexamethasone acetate	Sigma	D 1881	115H1008
vancomycin	Sigma	V 2002	118H0955
amikacin sulfate	Sigma	A 2324	37H602
etoposide	Sigma	E 1383	68H1001
quinine	Sigma	Q-1878	58H2505
verapamil hydrochloride	Sigma	V-4629	56H0925
5-fluorouracil	Sigma	F-6627	39H0901
poly-L-trytophan (MW 3900-4500)	Sigma	P-4647	84H5511
poly-L-trytophan (MW 9000-11500)	Sigma	P-0644	115F50011
cyclosporin A	Calbiochem	239835	B24596
amphotericin B	Sigma	A 4888	68H4111
tamoxifen	Sigma	T-5648	28H1033
cetrorelix acetate	Asta		Ber x 544

A known amount of each compound (determined using a Metler Analytical Balance Model AG245) was placed in a 10 ml scintillation vial. Two or three ml of PEG 400 (Kodak, Catalog # 1369941, Lot # 1156703112) were added to each vial using a micropipettor (a Gilson Pipetman, 1000 µl). Each vial was gently swirled to determine whether or not the drug had dissolved. If dissolution was less than complete, the vial was vortexed at a low speed. If vortexing failed to effect dissolution, the vial was sonicated in a water bath until the drug had dissolved. The time to dissolution was noted. Following dissolution, the sample was transferred to a cuvette and the absorption monitored using a UV/Vis spectrophotometer

(Model Lambda 3B, Perkin Elmer). All samples with the exception of trans-retinol and α-tocopherol were monitored for absorption in the 200-400 nm range.

The masses of each drug and the wavelength at which maximum absorption was measured are set forth in Table 2.

TABLE 2

COMPOUND	MASS USED (MG)	λ OF MAXIMUM ABSORBANCE (NM)
trans- retinol (vitamin A)	4.80	360
α-tocopherol (vitamin E)	11.25	280
dexamethasone	6.75	250
dexamethasone acetate	5.88	285
vancomycin	3.97	N/A
amikacin sulfate	9.82	N/A
etoposide	5.85	232/280
quinine	6.17	280
verapamil hydrochloride	6.51	280
5-fluorouracil	5.68	280
poly-L-trytophan (MW 3900-4500)	5.10	280
poly-L-trytophan (MW 9000-11500)	4.40	280
cyclosporin A	5.00	280
amphotericin B	4.30	N/A
tamoxifen	7.95	280
cetrorelix acetate	5.48	N/A

The relative solubility in PEG 400 and water is indicated in Table 3, below. In the table, "+" refers to a solubility in PEG 400 that is at least 1.5 times the solubility in water, "++" refers to

a solubility in PEG 400 that is at least ten times the solubility in water, and "+++" refers to a solubility in PEG 400 that is at least fifty times the solubility in water.

TABLE 3

COMPOUND	SOLUBILITY IN PEG COMPARED TO WATER
trans- retinol (vitamin A)	++
alpha tocopherol (vitamin E)	++
dexamethasone	+++
dexamethasone acetate	+++
vancomycin	---
amikacin sulfate	---
etoposide	+
quinine	++
verapamil hydrochloride	---
5-fluorouracil	+++
poly-L-trytophan (MW 3900-4500)	+
poly-L-trytophan (MW 9000-11500)	+++
cyclosporin A	++
amphotericin B	+
tamoxifen	+++
cetrorelix acetate	+

EXAMPLE 2

FORMULATION METHODOLOGY

100 mg of a PEG component (either a PEGylated phospholipid or branched PEG, 40 kD, Shearwater Polymers, Huntsville, AL) is dissolved in 10 ml t-butanol. The solution is heated over a 45-60°C hot water bath and subjected to sonication until the solution clarifies. The optional next component (free phospholipid or dispersing agent) is added in an amount as in Table 1 and sonication is applied again until the mixture clarifies. 10 mg of paclitaxel (Hauser Laboratories or Natural Pharmaceuticals) is then added, followed by heating and sonication as above. The mixture is flash frozen over liquid nitrogen and lyophilized on an ice-water bath for 4 hours followed by room temperature overnight to remove t-butanol. The final lyophilisate may be optionally microfluidized at about 15,000 psi and then lyophilized again for storage. The dry powder so obtained may be rehydrated in 1.0 ml saline.

EXAMPLE 3

ALTERNATIVE FORMULATION USING STAR-PEG (STAR-PEG 631, MW=154 KD, 15 ARMS, SHEARWATER POLYMERS, HUNTSVILLE, AL)

The protocol of Example 2 is duplicated using 100 mg of the star-PEG or branched PEG described above. No additional phospholipid is added in this case.

EXAMPLE 4

ETHANOL INJECTION IN WATER

1.6664 g of branched polyethylene glycol, MW 20,000, 4 branches (bPEG) is first dissolved in 20 ml of ethanol in a round bottom flask at approximately 35°C in a water bath with a rotor stirrer for approximately 15 min or until the bPEG has dissolved. This results in a clear solution to which the 332.7 mg of paclitaxel is added and dissolved under the same conditions. This is then taken up into a syringe and injected into a cold stirring solution of 638.6 mg of albumin and 1.6428 g of sucrose. This results in the instant formation of nanoparticles (less than 1μm). The suspension is immediately lyophilized.

Sucrose (20%), human serum albumin (2%) with sucrose (5%), sodium salt of taurocholic acid (5%) with sucrose (20%), and lipids (1,2 dioctanol-sn- glycerol-3-

phosphocholine, 1,2 dilauroyl-sn-glycerol-3-phosphocholine and 1,2 dipalmitoyl-sn-glycerol-3-phosphocholine) (2%) are some of the stabilizing agents that have been tested using this method.

EXAMPLE 5

ETHANOL IN METHYL T-BUTYL ETHER

Branched polyethylene glycol (1.6590 g) and paclitaxel (332.0 mg) were dissolved in ethanol as described in Example 4. The solution was then transferred to a dripping funnel and allowed to drip slowly into cold and stirring methyl t-butyl ether. This resulted in the instant formation of nanoparticles (less than 1μm).

EXAMPLE 6

CHOLIC SALTS AS STABILIZING AGENTS

a) 1.6579 g of branched polyethylene glycol, MW 20,000, 4 branches (bPEG) was first dissolved in 30 mL of t-butanol in a round bottom flask at approximately 55°C in a water bath with a rotor stirrer for approximately 15 min or until the bPEG had dissolved. This resulted in a clear solution to which the 329.5-mg of paclitaxel was added and dissolved under the same conditions. This solution was then freeze-dried and lyophilized. The white flaky powder that resulted was hydrated with a solution of 631.3mg sodium taurocholate and 1.6360 g of sucrose. The suspension was microfluidized for 15 min using the Model 110S, Microfluidics International Corp., Newton MA. This resulted in an almost clear (translucent) solution; the particle size was less than 50 nm.

b) The procedure described in part a) was applied using three other cholic acid salts (sodium cholate, sodium glycholate and sodium deoxycholate). The ratio of polyethylene glycol to paclitaxel was kept the same (5:1) and the amounts of the cholic acid salts and sucrose used was 2% and 5% respectively. This is similar to that used in Example 4. The particle size after microfluidizing was less than 1μm.

EXAMPLE 7

TWEEN® 80 (POLYOXYETHYLENE 20 SORBITAN MONOOLEATE)

Branched polyethylene glycol (1.6429 g) and paclitaxel (330.8 mg) were prepared as described in Example 4 above. After lyophilization the dry material was hydrated with a 1% solution of Tween® 80 (356.8 mg) and microfluidized. This resulted in particles less than 1μm.

EXAMPLE 8

IN VIVO EVALUATION -- MAXIMUM TOLERATED DOSE (MTD) STUDIES

(a) BMS TAXOL®:

Taxol® (Bristol Myers Squibb) was acquired from the University of Arizona Animal Care Center at 6 mg/ml. This solution was further diluted in saline to reach various concentrations needed in the experiment. Mice were injected with 500 μl in a slow bolus over 1.5 minutes.

The dosages for the experimental groups were as follows: 40 mg/kg; 30 mg/kg; 20 mg/kg; 10 mg/kg; and control saline. These animals were injected once a day for 8 days. The 40 mg/kg group died immediately after receiving the first injection. The 30 mg/kg and 20 mg/kg groups survived a single injection on day one but all of the animals in these groups died after receiving the injection on day two.

The 10 mg/kg groups all survived the entire 8 days of dosing and showed a delayed (1-2 minutes) severe response, i.e., they fell on their side, could not move, and had very labored breathing and occasional gasping for 5-10 minutes after which they slowly recovered. The animals were weighed daily until the final day. On the final day of dosing the animals were sacrificed by CO_2 asphyxiation. A blood sample was collected by heart puncture and split for cell count and liver function assays. The heart, liver, left kidney and spleen were collected and weighed from each animal. These tissues were placed in formalin for histological analysis.

The animal weights and tissue weights were analyzed statistically. There was no significant effect of Taxol® at 10 mg/kg/day on body weight when compared to the saline

control. There was a significant increase in spleen weight in the 10 mg/kg/day Taxol® group. The difference was between a mean spleen weight of 0.074 g for the saline group to 0.094 g for the 10 mg/kg/day Taxol® group with a p value < 0.015.

(b) Branched PEG/Paclitaxel:

A formulation of the invention was prepared with 5.3126 g branched PEG 40,000 ("PEG 40K"), 8 branches (Shearwater Polymers), 1.0022 g paclitaxel, 100 ml t-butanol and 2.6919 g sucrose.

The following procedure was carried out on each of the two flasks independently: The PEG 40K was dissolved in 100 ml t-butanol with heating and sonication at approximately 55-60°C. The solution was heated and sonicated until a clear solution resulted. The paclitaxel was added and the mixture was sonicated at the aforementioned temperature until a clear solution resulted (approximately 20 minutes). Each flask was then lyophilized overnight.

Each lyophilisate was then hydrated with 100 ml saline containing the quantity of sucrose indicated above. The mixtures were then heated in a water bath to 52°C. The contents of the two flasks were combined. Total theoretical amounts of material combined: 10.6162 g branched PEG 40K; 2.0271 g paclitaxel; and 5.3847 g sucrose. This combined batch was then microfluidized for 15 minutes at 14,000 psi.

Approximately 160 ml of sample was collected. Two approximately 80 ml batches were placed in a round bottom flask and lyophilized over 72 hours.

Paclitaxel content in the lyophilized material (dry powder) was determined by HPLC analysis as 0.078 mg/mg. Daily stock vials were then prepared each containing 768.5 mg of the aforementioned lyophilized formulation. This was dissolved in a total volume of 6 ml of water, making a 10 mg/ml paclitaxel solution.

Seven groups of 5 mice were given increasing dosages of diluted paclitaxel solution. The groups received either saline, BMS Taxol® 10 mg/kg, or the branched PEG 40K/paclitaxel solution as prepared above. Dosages were 20 mg/kg, 40 mg/kg, 60 mg/kg, 100 mg/kg and 140 mg/kg paclitaxel. The paclitaxel formulations (BMS Taxol® or PEG 40K/paclitaxel) were further diluted in saline to reach concentrations needed in the

experiment. Mice were injected with 500 µl in a slow bolus over 1.5 minutes. The mice were injected once a day for 8 days, a Monday through Thursday schedule.

The saline group had no response to injection on day 1 or 2. All mice in the BMS Taxol® group showed a delayed (1-2 minutes) severe response, i.e., they fell on their side, could not move, and had very labored breathing and occasional gasping for 5-10 minutes after which they slowly recovered on both day 1 and 2. All mice receiving 20, 40, and 60 mg/kg paclitaxel using the branched PEG 40K/paclitaxel formulation prepared above showed no response to the injections on day 1 or 2. 100 mg/kg paclitaxel resulted in slight distress, i.e., movements were slowed for 1-2 minutes but no breathing problems were observed on day 1 or day 2. The 140 mg/kg group showed moderate distress immediately after injection, i.e., they did not move and their breathing was slightly labored. Within this group one mouse died on day 1, one-hour post injection, and one mouse died two hours post injection. On day 2 one mouse died immediately after injection.

In summary, over three days, the average dose tolerance was found to be at least 10X in terms of toxicity tolerance for the branched PEG/paclitaxel formulation of the invention compared to Taxol®. By the end of two weeks all of the animals receiving 100 mg and 140 mg/kg/day of the branched PEG/paclitaxel formulation had died. All of the animals receiving 60 mg/kg/day and below survived, however. Compared to Taxol®, the results of this *in vivo* study support a ten-fold improvement in acute safety for the branched PEG/paclitaxel formulation of the invention and a six-fold improvement for subacute administration.

EXAMPLE 9

PREPARATION OF PACLITAXEL "MICROBUBBLES"

The formulation described in Example 2 is suspended in a 3 ml glass vial volume in a 1.6 ml saline solution, paclitaxel concentration 10 mg per ml, and the vial is sealed with a headspace of perfluorobutane gas. The vial is agitated at 4,500 rpm using a Capmix (ESPE, Morris, IL) dental amalgamator for 60 seconds. This results in microbubbles bearing paclitaxel.

EXAMPLE 10

TREATMENT USING THE PACLITAXEL MICROBUBBLES

A patient with prostate cancer receives an IV injection of the microbubbles described in Example 5, dose of paclitaxel = 30 mg. An endorectal ultrasound probe is placed in the patient's rectum and ultrasound, 1.0MHz, 1.0 Watt/cm^2, is applied across the patient's rectum for 30 minutes. The ultrasound probe is adjusted so that the energy is concentrated on the prostate gland. Ultrasound energy is adjusted so that the microbubbles are ruptured as they circulate within the patient's prostate gland. Increased delivery of paclitaxel to the cancer within the prostate is achieved. The patient is then treated with radiation therapy with a fraction of 300 Rads as part of a multi-dose treatment regiment. The paclitaxel acts as a radiation sensitizer and the ultrasound-mediated delivery enhances cellular uptake of the drug by the cancer.

EXAMPLE 11

TREATMENT OF A BREAST CANCER PATIENT

A patient with breast cancer may develop an allergic reaction to Taxol® due to the surfactants in the formulation. Still the patient will need the drug to control her disease and require pre-medication to avoid allergic reaction as well as sustained, slow infusion of the drug. The new formulation described in Example 3 is made available. Because of the absence of surfactants, the paclitaxel can be administered as an IV bolus injection without premedication.

EXAMPLE 12

COATED GRAFTS

Paclitaxel and branched PEG are dissolved in t-butanol at a drug concentration of 10 mg per ml and a weight ratio of branched PEG to the drug of approximately 5:1. This material is atomized and sprayed onto a Gortex® graft for coronary bypass. The graft is dried as the drug/branched PEG adsorbs on the surface of the graft. The resultant graft is implanted in a patient who needs a coronary artery bypass graft. Compared to grafts without this pretreatment there is a much higher rate of graft restenosis. The PEG adsorbed on the surface

retains the paclitaxel as a depot, releasing it over the proper time course to prevent fibrointimal hyperplasia.

EXAMPLE 13

SUSTAINED RELEASE DEPOT OF PACLITAXEL

Paclitaxel is dissolved in t-butanol at a concentration of 20 mg per ml with 100 mg per ml of branched PEG. The material is lyophilized and reconstituted at a concentration of 25 mg per ml paclitaxel in physiological buffered saline (PBS). The material appears as a viscous suspension of particles up to about an average size of 100 to 200 microns. This material is applied as a paste to the peritoneal surfaces of a patient with ovarian cancer following laparotomy and debulking for recurrent tumor. The formulation acts as a sustained release depot within the peritoneal cavity helping to control the patient's cancer.

EXAMPLE 14

SUSTAINED RELEASE DEPOT DELIVERY OF A PEPTIDE

The peptide leuprolide acetate is dissolved in t-butanol at a concentration of 20 mg per ml with 100 mg per ml of branched PEG. This is reconstituted with PBS at a drug concentration of 50 mg per ml. Two cc of this material is injected subcutaneously into a patient with prostate cancer. The sustained release of leuprolide acts to decrease hormonal stimulation and help control his cancer.

EXAMPLE 15

DERIVATIZATION OF POLYETHYLENE GLYCOL WITH PHOSPHATE MOIETIES

In a 1 liter round bottom flask is added 5 grams (125 micromoles, 1 equiv.) of 8-armed, branched polyethylene glycol (ω or terminally hydroxylated PEG, Shearwater Polymers, Huntsville Alabama) and 17.4 microliters of triethylamine (131.5 micromoles, 1.05 equiv., Aldrich, Milwaukee, Wisc.) in 250 ml dimethylformamide (DMF, Mallinckrodt, St. Louis, Mo.). The solution was cooled to 0° C in an ice bath. To this solution was added by dropwise addition, 20.2 mg (131.5 micromoles, 1.05 equiv.) of phosphorous oxychloride (Mallinckrodt, St. Louis, Mo.) in 5 ml of DMF. The mixture was allowed to equilibrate to

room temperature followed by stirring for an additional 8 hours. The solution was then quenched with 10 ml of water and the pH adjusted to neutrality.

The reaction mixture was then concentrated *in vacuo* followed by resuspension/dissolution in 100 ml of water. The product was dialyzed in a 1000 Molecular weight cutoff (MWCO) dialysis bag against deionized water. The solution from the bag was recovered, frozen, and lyophilized; yielding a white product containing mono-phosphorylated branched PEG.

If desired, the reaction may be repeated in the identical manner using twice, three times, four times, five times, etc., the stoichiometric amount of phosphorous oxychloride ($POCl_3$) to yield di-, tri-, tetra-, penta-, hexa-, hepta-, and per-phosphorylated branched PEGs. Note that the same procedure can be applied to any terminally hydroxylated branched PEG.

EXAMPLE 16

CATHETER COATING

A balloon dilatation catheter (Boston Scientific, Quincy, MA, model no. 13-188) is coated with a formulation of the invention, as follows: Branched PEG, MW=40,000 (Shearwater Polymers) is dissolved with paclitaxel (Natural Pharmaceuticals, Cambridge, MA) in t-butanol at a PEG concentration of 50 mg per ml and a paclitaxel concentration of 10 mg per ml. This material is atomized and deposited on the surfaces of the stent within a drying chamber. The balloon catheter is dried. The paclitaxel formulation appears as a white powder material coating the surfaces of the balloon. The catheter is then used for an angioplasty. The balloon is inflated at the site of vessel narrowing. The paclitaxel-containing coating impregnates the vessel wall as the balloon is inflated under high pressure. The local effects of the paclitaxel diminish fibroblast proliferation.

EXAMPLE 17

STENT COATING

A Wallstent® (Boston Scientific, Quincy, MA, model no. 42054) metallic stent is coated at described above. The coated stent is covered by a hazy white clathrate. The stent is advanced into the iliac artery of a patient with stenotic narrowing due to advanced

atherosclerosis. The stent is positioned and deployed at the site of stenosis using a Unistep Plus Delivery System (Boston Scientific). The paclitaxel aids in prevention of restenosis and the formulation releases the drug over a delayed period of time for maximal therapeutic benefit.

EXAMPLE 18

ALTERNATIVE STENT COATING

Another Wallstent® (same model) is treated with a gold plating process using electrochemistry to deposit a thin film of gold on the surface of the stent. A different branched PEG is prepared by substituting the terminal hydroxyl moieties of the PEG with thiol groups. The branched PEG is mixed with paclitaxel as described above in an organic solvent. The mixture is atomized and sprayed on the stent as described above in Example 12. Compared to the coating used in Example 12, the thiolated PEG used in this coating provides for a slower release rate, delivering the paclitaxel over a longer period of time.

EXAMPLE 19

The procedure of Example 14 is repeated with a poly(alkylene oxide) comprising 50% ethylene oxide monomers and 50% propylene oxide monomers. The incorporation of propylene oxide groups prolongs the release of the paclitaxel, providing therapy for a longer period of time.

EXAMPLE 20

PREPARATION OF NANOPARTICLES WITH BRANCHED PEG STABILIZED WITH ALBUMIN

Samples were prepared according to the procedure of Example I except that after the first lyophilization the samples were hydrated with solutions of different concentrations of human serum albumin. Microfluidizing then followed for 40 minutes. This was done to ensure that the suspension was homogenous and that the particle size was consistent. 1:5 bPEG 20K 4a was used as the determinant on how the variation of albumin affects the formulation.

TABLE 4

AMOUNT OF ALBUMIN AS W/W%	PARTICLE DIAMETER
0.5%	0.07-0.3μM
1%	0.03-0.2μM
2%	0.02-0.2μM

EXAMPLE 21

PREPARATION OF NANOPARTICLES WITH LINEAR PEG STABILIZED IN ALBUMIN

The procedure of Example 2 was used. The ratio of paclitaxel to PEG was maintained at 1:5 for PEG 35K, 20K & 10K. The difference between linear and branched PEG was not apparent without the use of human serum albumin. When the different formulations were suspended in an albumin solution, the difference in particle size was apparent and in the linear PEG the toxicity was greater.

TABLE 5

FORMULATIONS MADE USING LINEAR PEG W/HSA (2-5%)	SIZE
PEG 10K	> 1μM
PEG 20K	0.05-0.7μM
PEG 35K	0.05-0.7μM
BRANCHED PEG 20K & 40K	0.02-0.2μM

EXAMPLE 22

TABLE OF STABILIZING AGENTS AND RESULTS

The formulation used was of 1:5 paclitaxel to bPEG 20K 4a, prepared in the same way as described in Example 1. The stabilizing agents tabulated below were added to the formulation in the same manner as described for albumin in Example 16.

Table 6

COATING AGENT	RESULT (PARTICLE DIAMETER)
HUMAN SERUM ALBUMIN	20-300NM
POLYVINYL ALCOHOL	50-500NM
20% SUCROSE	<1μM BUT RE-AGGREGATE AFTER TIME
POLYVINYL PYRROLIDONE	200NM-1μM
METHYLCELLULOSE	<1μM
CARBOXYMETHYL CELLULOSE	<1μM
ALGINIC ACID	<1μM
PEG 400	<1μM
DEXTRIN	<1μM

EXAMPLE 23

NANOPARTICLES USING PHOSPHORYLATED PEG

The procedure of Example I was repeated (ratio of 1:5 paclitaxel: PEG) using a 2% albumin solution in a phosphate buffer (pH7.24) to hydrate the dry complex of the PEG and paclitaxel. This suspension was microfluidized for 45 minutes. No crystals were observed. Large particles resulted which appeared to aggregate.

EXAMPLE 24

EXAMPLES USING POLOXAMERS (PLURONICS) AS A STABILIZER FOR NANOPARTICLES

The procedure and conditions used were as described in Example I with the exception that Pluronic L64 (25%w/v) was dissolved into the t-butanol after the PEG. The paclitaxel was dissolved at a 1:5 ratio with PEG and the solution lyophilized. The resultant white, flaky powder was hydrated with an equivalent amount of a saline solution. The suspension was then placed in a flask with a rotor stirrer and immersed in a hot water bath set at 40° to 43° C. This was stirred for 45 min. No crystals were observed, but the suspension appeared to be more fluid than the PEG/paclitaxel suspension. If this formulation had been microfluidized or subjected to conditions where the particle size could have been altered, this would have resulted in a stable suspension of smaller particles (<1 μm). This is due to the surfactant nature of poloxamers.

EXAMPLE 25

THE COUPLING OF AN ACTIVATED PEG TO ALBUMIN

The PEG is initially activated using trichloro-s-triazine (TsT), which is a symmetrical heterocyclic compound containing three reactive acyl-like chlorines. PEG activation is necessary to form derivatives that are amine reactive where proteins linkages, in this case albumin, can develop. The activated PEG is slowly added to a solution of albumin in a 0.1 M sodium borate buffer at a concentration of 2-10 mg/ml. Note that at least a five-fold molar excess of the activated PEG should be present. This reaction takes 1 hr. at 4°C and the excess PEG can be removed by dialysis or gel filtration using a column of Sephacryl S-300 (Hermanson G.T, (1996) "Bioconjugate Techniques" Academic Press, San Diego).

EXAMPLE 26

IN VIVO MAXIMUM TOLERATED DOSE OF THE PEG/PACLITAXEL FORMULATION

A maximum tolerated dose (MTD) study was designed to test the safety of the stabilized paclitaxel formulation described in Example 16. These experiments were carried out in non-tumor bearing nude mice of between 6 and 8 weeks and an approximate body weight of 25 g. The animals were warmed under a heat lamp for 15 minutes and then placed

into a mouse restraint. An injection dose was then administered through the tail vein at a volume not exceeding 20 ml/kg. The maximum tolerated dose was defined to be the highest dose where the animals did not lose more than 10% body weight for two weeks after a single dose of drug. The mice were caged in groups of 5 for the holding period and were provided with food and water *ad libitum*. Animals completing the study were euthanized by CO_2 asphyxiation after the final weighing. A control study was carried out using Bristol-Myers Squibb's TAXOL® in this strain of mice.

A group of three nude mice were tested, each receiving different doses of the formulation: 150, 200, and 250 mg/kg. Their body weight was monitored following the injection and results are present in the figure below. In all tested mice, body weight change was approximately proportional to the dose given. The body weight decreased less than 10% of the initial weight, except for the mouse that received 250 mg/kg, (that mouse died on day 4). In the control study, the MTD of BMS TAXOL® was determined as 20 mg/kg. The single dose MTD for the formulation is 200 mg/kg and exceeds ten times that of BMS TAXOL®. (See Table 7 below.)

TABLE 7

FORMULATION	MAXIMUM TOLERATED DOSE *MG/KG*
PEG/Paclitaxel Complex	*200*
TAXOL®	*20*

EXAMPLE 27

IN VIVO EFFICACY OF THE PEG/PACLITAXEL FORMULATION

Efficacy experiments were carried out in athymic, nude mice of same age, between 6 and 8 weeks, with an approximate body weight of 25 g. The mice were implanted with two LS 180 human colon adenocarcinoma tumors per animal, with one tumor being implanted in each flank. Following tumor cell implantation, the animals were held until tumors were measurable. They then received the test article at doses that were based on MTD. The

animals were warmed under a heat lamp for 15 minutes and then placed into a mouse restraint. An injection dose was then administered through the tail vein at a volume not exceeding 20 ml/kg. Animals were provided with food and water *ad libitum*. Tumors were measured using a digital caliper. The weight of the tumor was calculated using the approximate equation of weight = (length x width^2)/2, where length and width is expressed in mm and weight in mg. Animals were euthanized by CO_2 administration when the estimated tumor weight exceeded 1 g.

The efficacy of the formulation was monitored following single administration at 2/3rd of MTD (maximum tolerated dose, 135 mg/kg) and full MTD (200 mg/kg), and compared to the efficacy of BMS TAXOL® administered at its MTD, i.e., 20 mg/kg.

Efficacy at 2/3^{rds} MTD. A significant tumor growth inhibition was seen after a single administration of the product of Example 16, i.e., at 135 mg/kg. Tumor inhibition was determined by calculating the minimum value of the T/C ratio defined as (mean tumor weight in treated group/mean tumor weight in control group) x 100%. According to NCl definitions, T/C ratios less than 42% indicate an active agent and T/C ratios below 10% indicate a highly active agent. The result of the 2/3^{rds} MTD testing is represented in Figure 7. A minimum T/C ratio of 8.5% was observed on day 10. Therefore, we conclude that the formulation of PEG stabilized paclitaxel at 2/3^{rds} MTD is a highly active agent.

Efficacy at Full MTD. Efficacy experiments were then carried out in the same model at full MTD, i.e., 200 mg/kg. Following the single administration, all tumors regressed throughout the duration of experiment. Tumor inhibition as defined above is not applicable here. Instead, tumor regression was calculated using the formula (1 - mean tumor weight/initial mean tumor weight) x 100%. A maximum tumor regression of 82% was seen on day 8. The body weight of the animals undergoing efficacy experiment was monitored and is shown in Figure 8. The maximum weight loss occurred eight days after injection. The animals fully recovered the initial weight within two weeks after injection. The same pattern was observed following the first and second injections; this suggests that the toxic effects are reversible.

CLAIMS

1. A pharmaceutical formulation comprising:

a matrix comprised of a spatially stabilized hydrophilic polymer that is optionally covalently bound to a phospholipid moiety;

a drug that is physically entrapped within the matrix but not covalently bound thereto, wherein the drug has greater solubility in polyethylene glycol 400 than in water;

an optional stabilizing agent;

an optional targeting ligand; and

an optional excipient.

2. The formulation of claim 1, wherein the hydrophilic polymer comprises a branched polymer.

3. The formulation of claim 2, wherein the branched polymer comprises an inner core structure attached to an outer structure, the inner core structure being more hydrophobic than the outer structure.

4. The formulation of claim 3, wherein the inner core structure is comprised of polypropylene oxide and the outer structure is comprised of members of the group consisting of polyethylene glycol and copolymers of propyleneoxide and ethyelenoxide, with the proviso that if copolymers of propyleneoxide and ethyelenoxide are present, the proportion of ethyleneoxide is greater than the proportion of ethyleneoxide in the outer structure.

5. The formulation of claim 2, wherein the matrix is comprised of a plurality of hydrophilic polymers that do not aggregate.

6. The formulation of claim 1, wherein the hydrophilic polymer is selected from the group consisting of polyethylene glycol, polyglycolide, polypropylene glycol, polyvinyl alcohol, polyvinyl pyrrolidone, polylactide, poly(lactide-co-glycolide), polysorbate, polyethylene oxide, polypropylene oxide, poly(ethylene oxide-co-propylene oxide),

poly(oxyethylated) glycerol, poly(oxyethylated) sorbitol, poly(oxyethylated) glucose), and derivatives, mixtures and copolymers thereof.

7. The formulation of claim 6, wherein the hydrophilic polymer is polyethylene glycol.

8. The formulation of claim 7, wherein the hydrophilic polymer is selected from branched polyethylene glycol, star polyethylene glycol, linear polyethylene glycol, and combinations thereof, optionally covalently bound to a phospholipid moiety, with the proviso that if the hydrophilic polymer comprises linear polyethylene glycol, a phospholipid moiety is necessarily covalently bound thereto.

9. The formulation of claim 7, wherein the polyethylene glycol is functionalized to contain at least one sulfhydryl, amino, lower alkoxy, carboxylate and/or phosphonate moieties.

10. The formulation of claim 7, wherein the polyethylene glycol contains a hydrolyzable linkage.

11. The formulation of claim 1, wherein the phospholipid moiety is a phosphorylated diacylglyceride.

12. The formulation of claim 11, wherein the phospholipid moiety is selected from the group consisting of dipalmitoyl phosphatidylethanolamine and 1-palmitoyl-2-oleylphosphatidyl-ethanolamine.

13. The formulation of claim 1, wherein the drug is at least about 1.5 times as soluble in polyethylene glycol 400 as in water.

14. The formulation of claim 13, wherein the drug is at least about ten times as soluble in polyethylene glycol 400 as in water.

15. The formulation of claim 1, wherein the optional stabilizing agent is present.

16. The formulation of claim 15, wherein the stabilizing agent is is selected from the group consisting of cholic acids and cholic acid salts.

17. The formulation of claim 16, wherein the cholic acid salt is selected from the group consisting of sodium tauracholate, sodium cholate, sodium glycholate and sodium deoxycholate.

18. The formulation of claim 15, wherein the stabilizing agent is a protein.

19. The formulation of claim 18, wherein the protein is selected from the group consisting of serum proteins, agglutination factors, peptide hormones, structural proteins, growth factors, metabolic potentiators, nuclear binding proteins, enzymes, antivirals, immunoglobins, and mixtures thereof.

20. The formulation of claim 19, wherein the protein is a serum protein.

21. The formulation of claim 20, wherein the serum protein is selected from the group consisting of albumin, recombinant albumin, defatted albumin, denatured albumin, arnylins, atrial natriuretic peptides, endothelins, endothelin inhibitors, urokinase, streptokinase, staphylokinase, vasoactive intestinal peptides, high density lipoproteins, low density lipoproteins, very low density lipoproteins, and mixtures thereof.

22. The formulation of claim 21, wherein the serum protein is selected from the group consisting of albumin, recombinant albumin, defatted albumin, denatured albumin and combinations thereof.

23. The formulation of claim 22, wherein the serum protein is defatted albumin.

24. The formulation of claims 22, wherein the serum protein is denatured albumin.

25. The formulation of claim 1, wherein the optional excipient is present.

26. The formulation of claim 25, wherein the excipient is a polyhydroxyalcohol.

27. The formulation of claim 26, wherein the excipient is selected from the group consisting of a free phospholipid, a saccharide, a liquid polyethylene glycol, propylene glycol, glycerol, ethyl alcohol, and combinations thereof.

28. The formulation of claim 27, wherein the excipient is a free phospholipid.

29.The formulation of claim 28, wherein the free phospholipid is selected from the group consisting of diacyl phosphatidylcholines, diacyl phosphatidylethanolamines, diacylphosphatidylserines, diacyl phosphatidylinositols, diacyl phosphatidic acids, phosphorylated diacylglycerides

30. The formulation of claim 29, wherein the free phospholipid is a phosphorylated diacylglyceride.

31. The formulation of claim 30, wherein the free phospholipid is selected from the group consisting of dipalmitoyl phosphatidylethanolamine, and 1-palmitoyl-2-oleoylphosphatidyl-ethanolamine.

32. The formulation of claim 28, wherein the phospholipid moiety bound to the hydrophilic polymer and the free phospholipid are the same.

33. The formulation of claim 28, wherein the phospholipid moiety bound to the hydrophilic polymer and the free phospholipid are different.

34. The formulation of claim 1, wherein the drug is an anti-cancer agent.